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Full Length Research Paper

Photosynthetic induction in leaves of two cucumber genotypes differing in sensitivity to low-light stress

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Low light is one of the most important stress factors affecting cucumber production in China greenhouse. Two cucumber genotypes (*Cucumis sativus* L.), Deltastar with low light-tolerance and Jinyan No. 2 with low light-sensitivity were used to study the response of gas exchange, chlorophyll fluorescence, stomatal opening and ribulose-1, 5-bisphosphate carboxylase/oxygenase (Rubisco) activity to low light during photosynthetic induction. In this experiment, cucumber plants were exposed to 75 to 100 μmol·m^{-2}·s^{-1} (control light 500 to 550 μmol·m^{-2}·s^{-1}) at 25 or 17°C (day/night) for 20 days. Photosynthetic induction was determined during cultivation after pre-dark 14 to 16 h. The results showed that there were stomatal and non-stomatal limitations (biochemical limitation) during induction of leaf photosynthesis. However, the biochemical limitation played a primary role in the early stage, but the stomatal limitation was predominant in the later stage during the whole photosynthetic induction. The time of net photosynthetic rate (P_N), stomatal conductance (g_s), actual photosystem II efficiency (Φ_{PSII}), linear electron transport rate (*J*) and non-photochemical quenching (NPQ) to reach maximal levels (steady-state) of cucumber leaves acclimated to low-light displayed longer induction process when compared with that under control light condition. Moreover, Jinyan No. 2, the low light-sensitive genotype, showed a longer start time of photosynthesis (STP), lower P_N, Φ_{PSII}, degree of stomatal opening and Rubisco activation state, as well as higher NPQ than those of Deltastar.

Key words: *Cucumis sativus*, low light, photosynthetic induction, chlorophyll a fluorescence, Rubisco, stomata.

INTRODUCTION

Light is an indispensable resource for plants growth and development where irradiance affects plant biochemical composition and morphology. Leaves grown under low-light are generally thinner when compared with those grown under high light, with a wider overall area and loosely packed mesophyll cells (Murchie et al., 2005). Moreover, changes also occur at chloroplast level. The number of chloroplasts decreases, while the size of chloroplasts, the number of grana and grana lamellae increase in low-light-grown leaves (Lichtenthaler and Burkart, 1999). In comparison, leaves grown under high irradiance often have higher rates of photosynthesis due to a higher ribulose-1, 5-bisphosphate carboxylase /oxygenase (Rubisco) carboxylation activity and components of electron transport and ATP synthesis. Thus, high light responses occur to maximize light-saturated rates of photosynthesis, while the low-light responses occur to enhance the efficiency of photon capture (Murchie et al., 2005).

Moreover, the stated differences between high and low-light-acclimated leaf exist not only between species, but also within species (Demmig-Adams and Adams, 1994).

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The greenhouse varieties of horticultural crop such as cucumber (*Cucumis sativus* L.) belong to an ecological type naturalized and selected for a low-light and/or lower temperature environment of winter and spring seasons. These varieties therefore have a higher hereditary adaptability to low light and/or lower temperature than open-field varieties (Zhou et al., 2006; Li et al., 2009). In general, tolerant genotypes maintained greater rates of growth, stronger photosynthesis capacity and lower losses of plant productivity than the sensitive genotypes under low light conditions.

When the plant is shifted from darkness to light, net photosynthetic rate (P_N) in photosynthetic organs (or organelles) increases gradually and the phenomenon of photosynthetic induction is named the 'lag phase' of photosynthesis, which existed in all tissues, such as intact leaves, leaf fragments, mesophyll cells, protoplasts and chloroplasts of a wide range of crop. This phenomenon occurs as a result of slow opening of the stomata, activation of enzyme and raising the level of photosynthetic. Calvin-cycle intermediates are depleted in the preceding dark period after illumination (Xu, 2002). Accordingly, three phases of photosynthetic induction can be distinguished (Urban et al. 2007) as follows: (1) During the first 1 to 2 min of leaf exposure to irradiance, activities of enzymes involved in the regeneration of the primary CO_2 acceptor RuBP and other enzymes increase (Kirschbaum and Pearcy, 1988); (2) Incomplete activation of Rubisco, catalyzing the primary carboxylation reactions, is considered the key biochemical limitation during most of the induction period (Woodrow and Mott, 1989; Mott and Woodrow, 2000); (3) The opening of stomata is the slowest step in the photosynthetic induction process (stomatal limitation) and can take over an hour to reach full induction (Kirschbaum et al., 1998; Allen and Pearcy, 2000). Many factors influence photosynthetic induction kinetics. Above all, the plant acclimation to the growth environment, especially the previous light (low and high-light) history leads to biochemical and anatomical changes in leaves and affects the rate of photosynthetic enzyme activation. In addition, several factors including physiological, ecological and experimental conditions can also cause different lengths of photosynthetic induction phase (Urban et al., 2007; Naramoto et al., 2001; Padmasree and Raghavendra, 1999; Portes et al., 2006)

Cucumber is an important vegetable crop grown under greenhouse condition in winter and spring seasons in China. Low light is one of the most important limiting factors affecting the cucumber production. During winter production of cucumber, the coverings on the greenhouse are removed as early as possible in the morning in order to shorten the start time of photosynthesis (the time of P_N from zero to maximum, STP) and low light irradiance is used effectively. The fast photosynthetic induction after leaf illumination contributes to the prolonged duration of maximum photosynthetic rate and enhances carbon fixation. However, the change in the photosynthetic rate, photochemical efficiency and capacity of energy distribution in light induction process of cucumber in low light-acclimated leaves remain unclear.

In this study, two genotypes of cucumber with different low light tolerance were used to study the changes in photosynthetic induction affected by low light and the genotypic differences in photosynthetic response to low light during induction process.

MATERIALS AND METHODS

Plant materials and growth conditions

Two cucumber cultivars Deltastar (a low light-tolerant genotype for cultivation in greenhouses, from Rijk Zwaan Corporation, Holland) and Jianyan No. 2 (JY2, a low light-sensitive genotype for cultivation in the open field, from Tianjin Cucumber Institute, China) were pre-cultured in a phytotron. Seeds were sown directly in 12 cm plastic pots containing a mixture of peats and vermiculite (v/v, 2:1). All the plants were grown under controlled light intensity (CT: 500 to 550 $\mu mol \cdot m^{-2} \cdot s^{-1}$) until they reached 2 to 3 leaf stages and then half of the plants were transferred to low light (LL: 75 to 100 $\mu mol\ m^{-2} \cdot s^{-1}$) and the remaining half kept at the controlled intensity, that is, JY2 low light treatment (LL-JY2), JY2 control (CT-JY2), Deltastar low light treatment (LL-Deltastar) and Deltastar control (CT-Deltastar), respectively. Photoperiod was 10 or 14 h (day/night) and temperature was maintained at 25 or 17°C (day/night). After 20 days of cultivation, the third unfolded leaves (from the plant top) were sampled for gas exchange parameters, chlorophyll a fluorescence parameters, scanning electron microscopy (SEM) and Rubisco activity analysis after dark adaptation for 14 to 16 h with four or five replicates.

Photosynthesis

Photosynthetic gas exchange parameters were measured using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE, USA). The P_N, stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) of leaves were determined based on the growing conditions of seedlings, that is, PPFD was 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LL) and 500 $\mu mol \cdot m^{-2} \cdot s^{-1}$(CT), respectively. Assimilation chamber CO_2 concentration, air temperature and relative humidity (RH) were maintained at 400 ± 10 $\mu mol \cdot mol^{-1}$, 25 ± 1°C and 50 to 60%, respectively. The airflow rate in the assimilation chamber was maintained at 500 $\mu mol \cdot s^{-1}$. The data for P_N, g_s and C_i were automatically recorded every three minutes for each circle for 15 circles. Stomata limitation (Ls) was calculated as $1-C_i / C_a$, where C_i is the intercellular CO_2 concentration and C_a is the ambient CO_2 concentration (Berry and Downton, 1982).

Chlorophyll a fluorescence

Chlorophyll (Chl) a fluorescence parameters were measured simultaneously using a LI-6400 portable photosynthesis system equipped with a 6400 - 40 fluorometer on the same leaves previously used for gas exchange measurement. After dark adaptation of samples for 14 to 16 h, the minimal fluorescence (F_o) was measured under weak modulated light (<0.1 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Then, a 0.8 s saturating flash (>7000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) was applied to determine the maximum Chl fluorescence yield (F_m) and F_v/F_m (F_v, the variable Chl fluorescence yield, is defined as F_m-F_o), where F_v/F_m is the maximum quantum yield of photosystem II (PSII) or maximal efficiency of PS II photochemistry. Following this, the leaves were continuously illuminated with a red-blue actinic light at 500 $\mu mol \cdot$

$m^{-2}·s^{-1}$ (CT) and 100 μmol·$m^{-2}·s^{-1}$ (LL), respectively. Under actinic light, the steady-state value of fluorescence (F_s) was recorded and saturating pulse (>7000 μmol·$m^{-2}·s^{-1}$) was imposed at 30 s intervals to determine the maximum fluorescence level in the light-adapted state (F_m'). The actinic light was removed and the minimum fluorescence level in the light-adapted state (F_o') was determined by illuminating the leaf with a 3 s pulse of far-red light. After the far-red illumination, actinic light was switched on again to begin a new measurement cycle. All the fluorescence was detected from the leaf's adaxial side.

Other fluorescence parameters, including the photochemical quantum yield of PS II or the actual PS II efficiency under irradiance ($\Phi_{PS\ II}$), non-photochemical quenching (NPQ) and the linear electron transport rate (J) were calculated from the following equation (Genty et al., 1989; Bilger and Björkman, 1990).

$$\Phi_{PSII} = (F_m' - F_s)/F_m'$$
$$NPQ = F_m/F_m' - 1$$
$$J = (F_m' - F_s)/F_m' \times PPFD \times f \times a_{leaf}$$

Where, PPFD is incident photosynthetic photon flux density, f is the fraction of absorbed quanta that is used by PS II, taken as 0.5 for C_3 plants and α_{leaf} is the total leaf absorbance in the visible range, taken as 0.85 usually.

Stomata measurements

After dark adaptation of samples for 14 to 16 h, the 3rd unfolded leaves of these plants were illuminated continuously with PPFD of 500 μmol·$m^{-2}·s^{-1}$ (CT) and 100 μmol·$m^{-2}·s^{-1}$ (LL), respectively, as seedling actual growth condition. The irradiation lasted for 0, 5, 10, 25 and 45 min, respectively, after which the leaf discs (2 mm × 2 mm), were cut and fixed immediately with 2.5% (v/v) glutaraldehyde and subsequently dehydrated in increasing ethanol/water mixture up to pure ethanol. After fixation and dehydration, the samples were dried using carbon dioxide. Later the samples were fixed to aluminum stubs with double-sided adhesive tape and sputter-coated with gold. Observation was done in Hitachi S-570 scanning electron microscope (SEM) (Hitachi Ltd., Tokyo, Japan) operating at 20 kV. The degree of stomata transversal or longitudinal opening in upper and lower epidermis of leaves (after irradiation which lasted for 0, 5, 10, 25 and 45 min, respectively) from 10 to 15 microscope fields per treatment was measured and pooled together for statistical analysis.

Meanwhile, the stomata in a typical microscope field at different irradiation period were photographed using a digital camera attached to the SEM. The guard cell sizes (length × width) of leaves (at irradiation 45 min) on both leaf epidermises were measured and the stomata number per microscope field was quantified to obtain stomata density and total number of stomata per leaf.

Rubisco activity analysis

Under the same growing conditions, leaf discs were cut after irradiation for 0, 5, 10, 25 and 45 min and were immediately frozen using liquid nitrogen. The samples were then homogenized using a pre-cooled mortar and pestle in cold extraction buffer containing 50 mM Tris-HCl (pH 7.5), 1mM EDTA, 1 mM $MgCl_2$, 12.5% (v/v) glycerine, 10% polyvinylpyrrolidone (PVP) and 10 mM β-mercaptoethanol. The homogenate was centrifuged at 15,000 × g for 15 min at 4°C.

Rubisco activity was measured spectrophotometrically by coupling 3-phosphoglyceric acid formation with NADH oxidation at 25°C, following the method described by Zheng (2006). The total activity was assayed after the crude extract was activated in a 0.1 ml activation mixture containing 33 mM Tris-HCl, pH 7.5, 0.67 mM EDTA, 33 mM $MgCl_2$ and 10 mM $NaHCO_3$ for 10 min. Initial Rubisco activity measurements were taken in a 0.1 ml reaction medium containing 50 mM Hepes-KOH (pH 8.0), 10 mM $NaHCO_3$, 20 mM $MgCl_2$, 2.5 mM dithiothreitol (DTT), 1 mM EDTA, 10 U of creatine phosphokinase, 10 U of 3-phosphoglyceric phosphokinase, 10 U of glyceraldehydes 3-phosphate dehydrogenase, 5 mM ATP, 0.15 mM $NADH_2$, 5 mM phosphocreatine, 0.6 mM ribulose-1,5-bisphosphate (RuBP) and 10 μl of extract. The change in the absorbance at 340 nm was monitored for 60 s. The ratio of initial to total activity was termed as the activation state of Rubisco, which estimated the degree at which the enzyme is carbamylated (Cen and Sage, 2005).

Statistics

The data were then entered into Microsoft excel 2003 spreadsheet and each value in figures or tables represents mean of 4 to 5 replications of measurements and S.D. The graphs were processed using Microsoft excel 2003. Data were subjected to analysis of variance using SPSS statistical package (10.0 for Windows). Significant differences between the two genotype plants were reported at $P < 0.05$, if not indicated otherwise.

RESULTS

Changes in gas exchange parameters during photosynthetic induction

The P_N of cucumber leaves increased rapidly during the first 12 or 15 min of illumination, then gradually reached to the maximum and later remained steady in plants shifted from the darkness to CT (PPFD 500 μmol·$m^{-2}·s^{-1}$) or LL (PPFD 100 μmol·$m^{-2}·s^{-1}$), respectively (Figure 1A). However, the P_N in dark-adapted leaves grown under low light increased slowly when compared with the leaves grown under CT light during the first few minutes of illumination. The P_N of leaves of JY2 and Deltastar cucumber grown under CT light reached maximum (8.39 and 7.19 μmol CO_2·$m^{-2}·s^{-1}$) on the 21st and 24th min, respectively. However, the STP in low-light-leaves was longer than that of the CT light leaves ($P < 0.05$). The STP for Deltastar (P_N 2.49 μmol CO_2·$m^{-2}·s^{-1}$) and JY2 (P_N 1.67 μmol CO_2·$m^{-2}·s^{-1}$) was 36 to 39 and 45 min, respectively. It also showed that JY2 required longer time to reach photosynthetic steady-state than Deltastar under low light condition. Changes in the g_s (Figure 1B) showed similar trends to that of P_N, but it rose slower than P_N during photosynthetic induction period. The g_s of cucumber leaves under CT light growth reached maximum between 36 to 39 min, while that under low-light reached maximum between 39 to 45 min after illumination. This suggested that the stomata opening might be affected by low light.

The C_i decreased gradually and declined to its minimum level at 18 min (marked with point 'S') in both cultivars under CT light condition, but under low light during early illumination, the decline was observed at 24 and 27 min (marked with point 'S') for Deltastar and JY2, respectively (Figure 1C). After the initial decline, the C_i values were steady. Meanwhile, L_s increased gradually to the maximum at 18 to 27 min (marked with point 'S'),

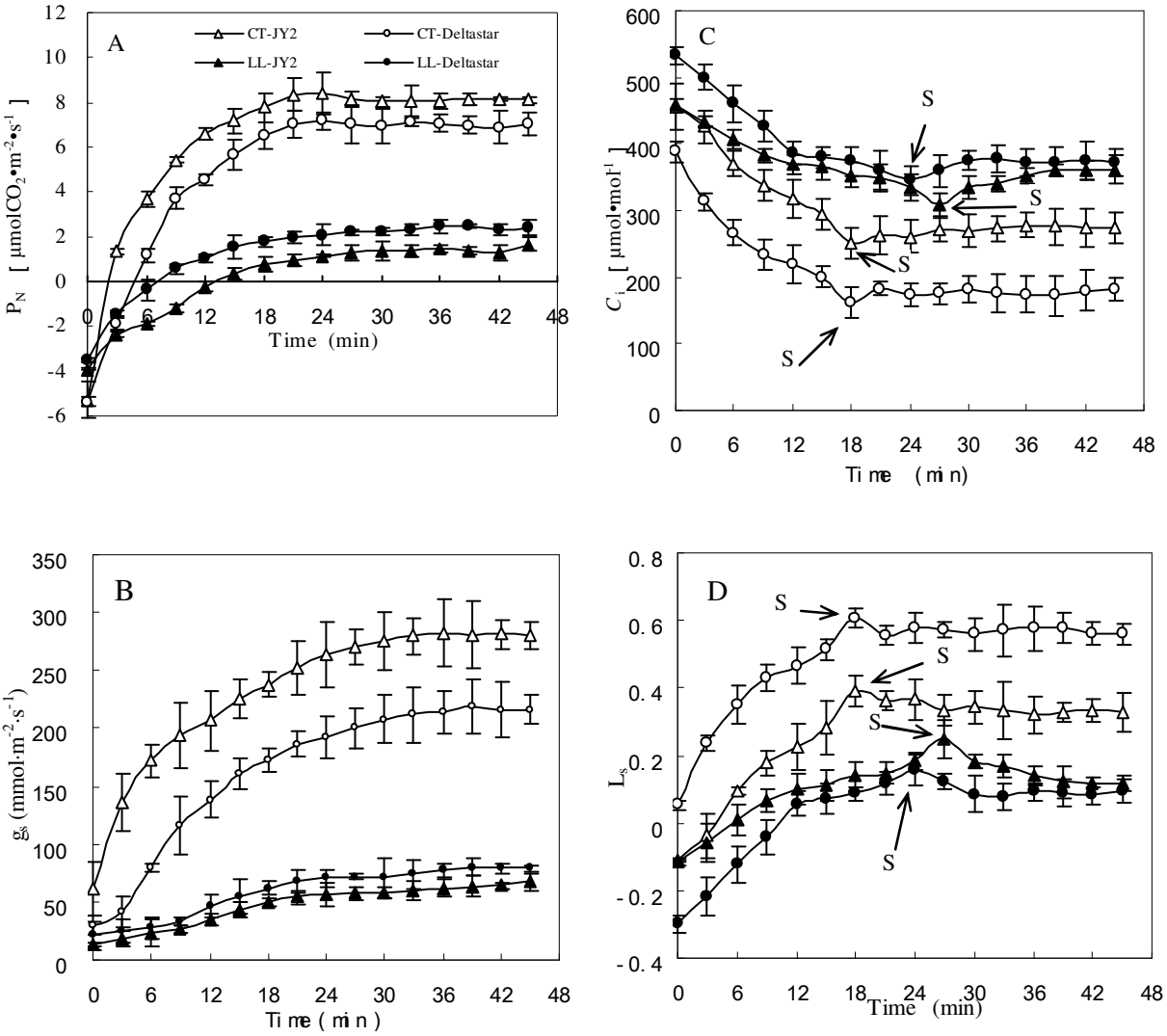

Figure 1. Changes of net photosynthetic rate, P_N (A), stomatal conductance, g_s (B), intercellular CO_2 concentration, C_i (C), and stomata limitation value, L_s (D) of cucumber leaves during photosynthetic induction. Measurements were made under 500 μmol·m^{-2}·s^{-1} (CT) and 100 μmol·m^{-2}·s^{-1} (LL) irradiance, respectively, in ambient CO_2 (about 400 μmol·mol^{-1}) at 25 ℃. The different timing of the C_i and L_s changeover time points were marked with point 'S', which means two stage of photosynthetic induction, that is the early and later stage. Means ± S.D. (n = 4).

then slightly decreased (Figure 1D).

Changes in Chl a fluorescence parameters during photosynthetic induction

During the first few minutes of illumination, the values of Φ_{PSII} was very low (Figure 2A), but the fluorescence maximum (F_m') in the plants grown in light, were the highest (Figure 2C). The dissipation of excessive energy was re-emitted furthest as Chl fluorescence. Thereafter, PSII reaction centers were opened partially and excited stated electrons were transported away from PSII (Figure 2E) (Maxwell and Johnson, 2000). This resulted in the decrease of the maximum variable fluorescence in light-adapted state (F_m') (Figure 2C) and steady-state fluorescence under irradiance (F_s) (Figure 2D). Meanwhile, Φ_{PSII} increased gradually until it reached steady-state value. As illumination time prolonged, excessive light energy was converted into heat, that is, the value of NPQ gradually increased (Figure 2B).

After Chl fluorescence reached steady-state, Φ_{PSII} of Deltastar and JY2, increased approximately by 89.9% and 60.4% under low-light cultivation, respectively, compared with those under CT cultivation. This indicates that the efficiency of light energy captured was relatively higher in low light-acclimated leaves than in control light-acclimated leaves. Accordingly, it was a physiological

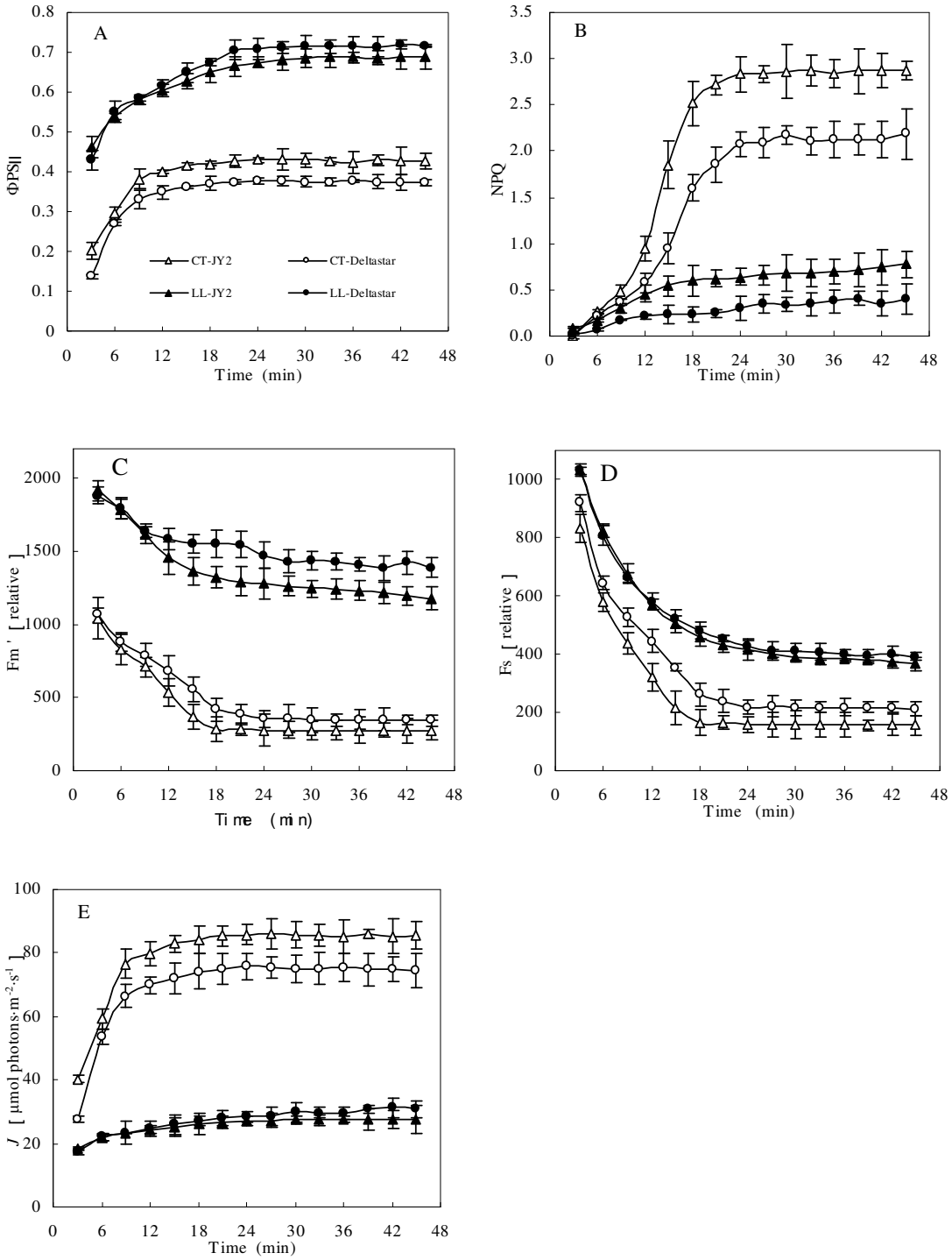

Figure 2. Changes of Chl a fluorescence in leaves of JY2 and Deltastar during photosynthetic induction. (A), Φ_{PSII}, (B), NPQ, (C), F_m', (D) F_s, (E) *J*. The actinic light was 500 μmol·m^{-2}·s^{-1} (CT) and 100 μmol·m^{-2}·s^{-1} (LL), respectively. Means ± S.D. (n = 4)

response for plant to acclimate itself to low light environment.

When cucumber plants were exposed suddenly to their actual light condition after dark acclimation for 16 h, Φ_{PSII}, NPQ and *J* of cucumber leaves under CT light growth increased to maximum levels within a short period (about 21 to 24 min), whereas, it took a little longer time (after 30 mins of illumination) for those under low light growth

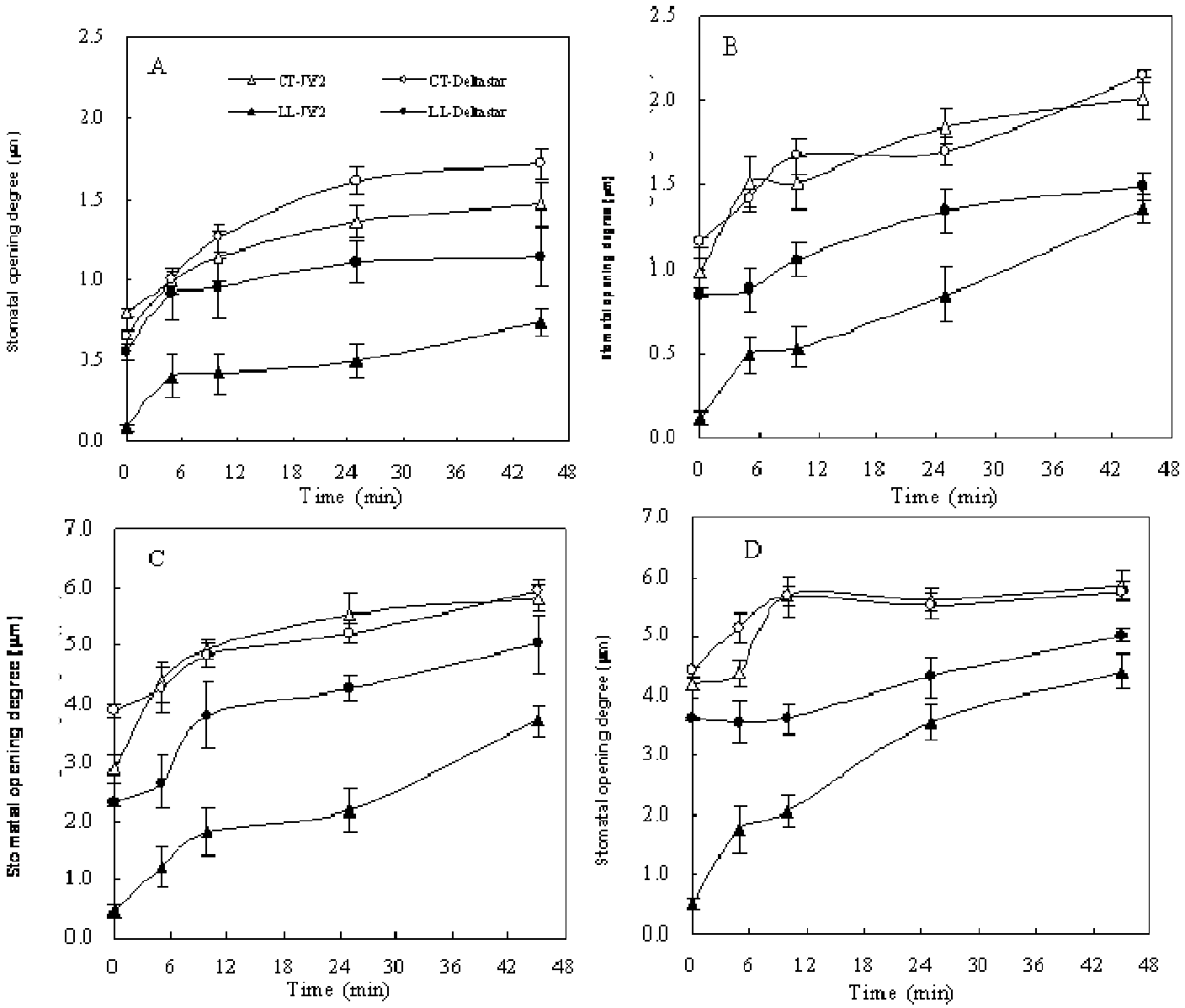

Figure 3. Changes in the degree of transversal stomata opening in upper epidermis (A) and in lower epidermis (B), longitudinal stomata opening in upper epidermis (C) and in lower epidermis (D) of cucumber leaves during photosynthetic induction. Measurements were made under 500 μmol·m^{-2}·s^{-1} (CT) and 100 μmol·m^{-2}·s^{-1} (LL) irradiance respectively after full dark-adapted for 14~16h. Means ± S.D. (n = 5).

(Figure 2). However, there were no significant differences in induction time in the Φ_{PSII}, NPQ and *J* between JY2 and Deltastar under low light cultivation.

Changes in the degree of stomatal openings during photosynthetic induction

Illumination affected stomatal opening. The degree of transversal stomata opening (Figure 3A,B) and the longitudinal opening (Figure 3C,D) in the upper (Figure 3A,C) and the lower epidermis (Figure 3B,D) all increased gradually during photosynthetic induction. It was evident from the observations of the stomata in JY2 (Plate I) using scanning electron microscopy and the changes of stomata in that Deltastar were similar to JY2 (data not shown). Meanwhile, the degree of stomatal opening in the cucumber leaves grown under low light was significantly smaller than the plants grown under CT light (Figure 3 and Plate I). After 45 min illumination under low light cultivation, the degree of transversal stomata opening in both upper or lower epidermis decreased by 33.3 or 30.3% in the case of Deltastar and 49.4 or 32.3% in JY2 (Figures 3A,B), respectively. Although, there were no significant differences between the two genotypes under CT light, the degree of stomatal openings of

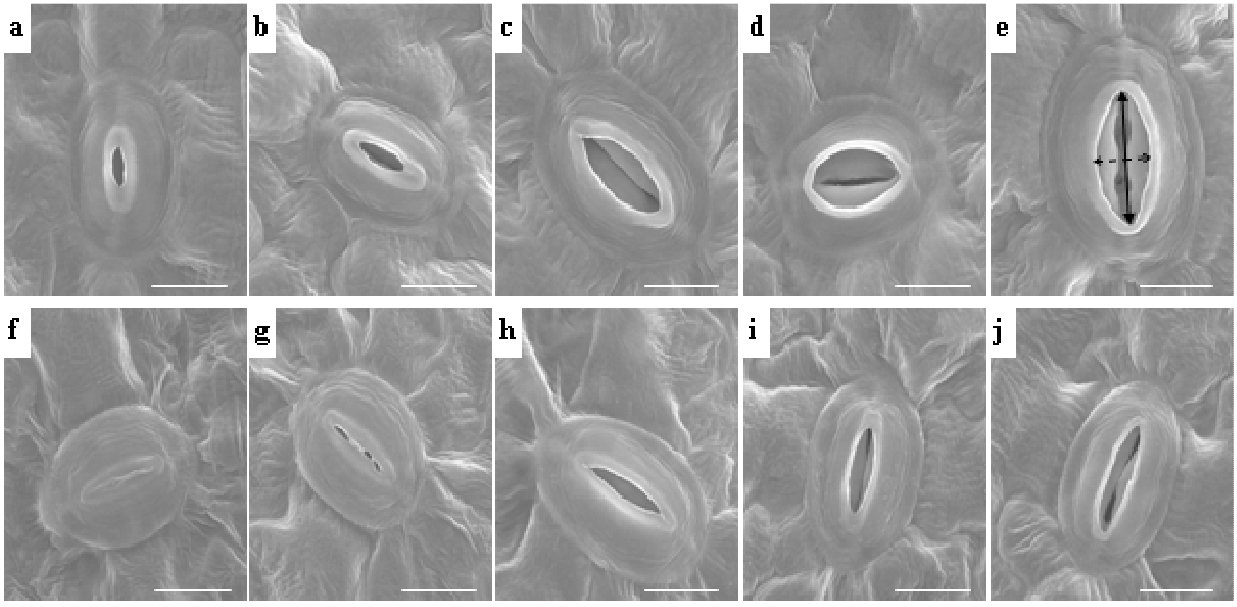

Plate 1. Changes in the degree of stomata openings on lower epidermis of Jinyan no.2 (JY2) leaves after irradiation that lasted 0, 5, 10, 25 and 45 min respectively scanned by electron microscope. (a - e), JY2 under 500 µmol·m^{-2}·s^{-1} (CT) after irradiation for 0, 5, 10, 25, and 45 minutes, respectively; (f - j) JY2 under 100 µmol·m^{-2}·s^{-1} (LL) after irradiation for 0, 5, 10, 25 and 45 min, respectively. ◄——► longitudinal distance, ◄- - -► transverse distance; Bars= 6.5 µm.

Deltastar leaves was significantly larger ($P < 0.05$) than that of JY2 under low light conditions (Figure 3).

The guard cell size on both upper and lower epidermis of cucumber leaves grown under low light condition decreased significantly ($P < 0.05$) than the plants grown under CT light condition (Table 1). The stomata density on the lower epidermis of leaves was distinctly larger than that of the upper epidermis in same light condition. The stomata density on lower epidermis of low-light grown leaves and the stomata number per leaf decreased greatly. They decreased by 37.1 and 40.3% in JY2 and by 15.3 and 8.7% in Deltastar under low light treatment for 20 days when compared with respective control (Table 1). It indicates that the low light intensity has distinct effect on the stomata development, especially in the low light-sensitive variety JY2.

Changes in Rubisco activity during photosynthetic induction

During photosynthesis induction, the initial Rubisco activity (Figure 4B) and the Rubisco activation state (Figure 4C) of cucumber leaves increased rapidly, but the total Rubisco activity (Figure 4A) changed slightly, especially in the first few minutes of illumination. The total Rubisco activity, the initial Rubisco activity and the activation state of Rubisco of cucumber leaves under low light treatments for 20 days were lower than under CT conditions. Under low light condition, the obvious differences ($P > 0.05$) could not be found in the total and initial activity of Rubisco between JY2 and Deltastar but the activation state of Rubisco in Deltastar was significantly higher ($P < 0.05$) than that in JY2. At the end of photosynthetic induction, the activation state of Rubisco in the leaves of Deltastar and JY2 under low light treatments was 83.5 and 58.8% of corresponding CT.

DISCUSSION

Photosynthetic limitations include stomatal and non-stomatal limitations. In this study, two stages of inductionprocess (the early and the later stage with marked 'S' in Figure 1) were identified and discussed to determine the role of stomatal limitation in photosynthetic induction of cucumber leaves grown under low light. In the early stage of induction, the increase of g_s and P_N were accompanied with L_s increase and C_i decrease. However, C_i level was high throughout this stage than the photosynthetic steady-state (later stage). This result indicated that, gradual increase in the P_N was not due to the increase in g_s, but due to the increase in the level of intermediates in the photosynthetic Calvin-cycle and gradual activation of enzymes involved in carboxylation reactions (Xu, 2002). It was consistent with the rapid increase in the initial Rubisco activity and the Rubisco activation state that was considered as the key enzyme in carboxylation reactions (Figure 4). In the later stage of induction, the g_s and P_N continued to increase, but L_s decreased while C_i was steady (Figure 1). These results

Table 1. Effect of low light on guard cell size, stomata density, and stomata number per leaf in upper or lower epidermis of cucumber leaves at photosynthetic induction steady state. Different letters in a column for same material mean significant difference at 5% level between treatments. Means ± S.D. (n=5).

Variety	Treatment		Guard cell size (length × width) (μm × μm)	Stomata density (number/mm^2)	Stomata number per leaf (×10^6·leaf^{-1})
Jinyan No.2	upper epidermis	CT	14.50 (± 0.12)×9.75 (± 0.09)^a	425 ± 17^a	4.463 ± 0.351^a
		LL	14.29 (± 0.02)×9.45 (± 0.10)^b	309 ± 18^b	2.936 ± 0.112^b
	lower epidermis	CT	14.63 (± 0.24)×10.23 (± 0.32)^a	1036 ± 22^a	10.374 ± 0.102^a
		LL	14.09 (± 0.16)×9.37 (± 0.17)^b	652 ± 36^b	6.194 ± 0.137^b
Deltastar	upper epidermis	CT	14.89 (± 0.45)×10.46 (± 0.33)^a	318 ± 24^a	2.862 ± 0.339^a
		LL	14.23 (± 0.08)×9.36 (± 0.14)^b	283 ± 18^a	2.745 ± 0.097^a
	lower epidermis	CT	14.89 (± 0.14)×10.47 (± 0.15)^a	825 ±41^a	7.425 ± 0.287^a
		LL	13.55 (± 0.22)×8.82 (± 0.52)^b	699 ± 27^b	6.780 ± 0.105^b

indicated that an increase in P_N was attributed mainly to the increase in g_s. Accordingly, the continuous increase in the degree of stomatal opening was seen in the middle and later stage of induction (Figure 3, Plate I). However, the changes in the enzyme activation such as Rubisco for biochemical limitation did not show significant difference at this stage (Figure 4). It was proposed that, there was indeed stomatal limitation during induction of leaf photosynthesis not only in the CT-light but also in low-light condition. However, non-stomatal limitation (biochemical limitation) could have played a greater role in the early stage and stomatal limitation was predominant in the later stage during cucumber photosynthetic induction.

When a plant is suddenly transferred from darkness to light, the stomatal opening, Rubisco activity and P_N of leaves are altered gradually to acclimate to the light change but there are some differences in the response rate, which possibly relates to anatomical structure and physiological function of leaves grown under different light environments (Yang et al., 2005). Sims et al. (1998) found that the length of photosynthetic induction period (photosynthetic induction time) lied mostly on how irradiance promoted the activation of Rubisco and opening of stomata. Rubisco activity is regulated by Rubisco activase and ATP supply that depended on electron transport activity (Hidema et al., 1991). It was possible that, a decrease in the carboxylation efficiency had been caused by a decline in electron transport (Figure 2E), low ATP production and low Rubisco activation state (Figure 4C) due to low light stress. This phenomenon was more obvious in low light-sensitive genotype JY2 than in low light-tolerant genotype Deltastar. Consequently, the time for photosynthetic induction under low light conditions increased when compared with CT light conditions.

The opening of stomata is likely to depend on the structure of guard cell and cell wall characteristics (Pessarakli, 1997). In this study, the guard cell size of cucumber leaves grown under low light conditions was less when compared with the plants grown under CT conditions (Table 1). Furthermore, the degrees of stomatal opening in the low-light leaves were dramatically lower than that of CT-light leaves in the whole process of photosynthetic induction, especially in the low light-sensitive genotype JY2 (Figure 3). These results indicated that low light intensity probably affected the stomata development. Hence, the regulating adaptability of open and close function by stomata to light environment was shortened and the photosynthetic induction time or the lag period prolonged. Moreover, several studies have shown that the stomata density of the low-light leaves or shade-leaves was much lower than that of the leaves under high-light or sun-leaves (Bergmann and Sack, 2007). The results (Table 1) in this study were consistent with the previous findings. The decrease in the stomata density was potentially related to the decline of stomata numbers per leaf (Table 1) and the increase of leaf areas (data not shown) under low light environment. The sensitivity of stomata to light intensity for low-light leaves was lower than that for high-light leaves, which may also relate to the lower stomata density in the low-light leaves (Zhang et al., 2002).

Chl a fluorescence could be used as a probe to study photosynthesis. In the experiments, Φ_{PSII} and NPQ of cucumber leaves grown under CT light reached maximum maximum (steady) levels in a short period and F_m' and F_s quickly decreased to steady-state. Whereas, more time was required for these Chl fluorescence parameters to reach steady state in low-light leaves (Figure 2). Duringlight induction of fluorescence quenching, the differences between the leaves grown under CT and low light irradiance were related to the start-up speed of corresponding dark photosynthetic reaction and response to stomata conductance. Moreover, it was also related to the size of xanthophylls cycle pools and transformation rate of xanthophylls cycle components from violaxanthin (V) through antheraxanthin (A) to zeaxanthin (Z) (Yang et al., 2005). The g_s and P_N of cucumber leaves removed from dark to CT light reached maximum value in a short time during photosynthetic induction. Hence, the leaves could start up Calvin cycle promptly to consume ATP and

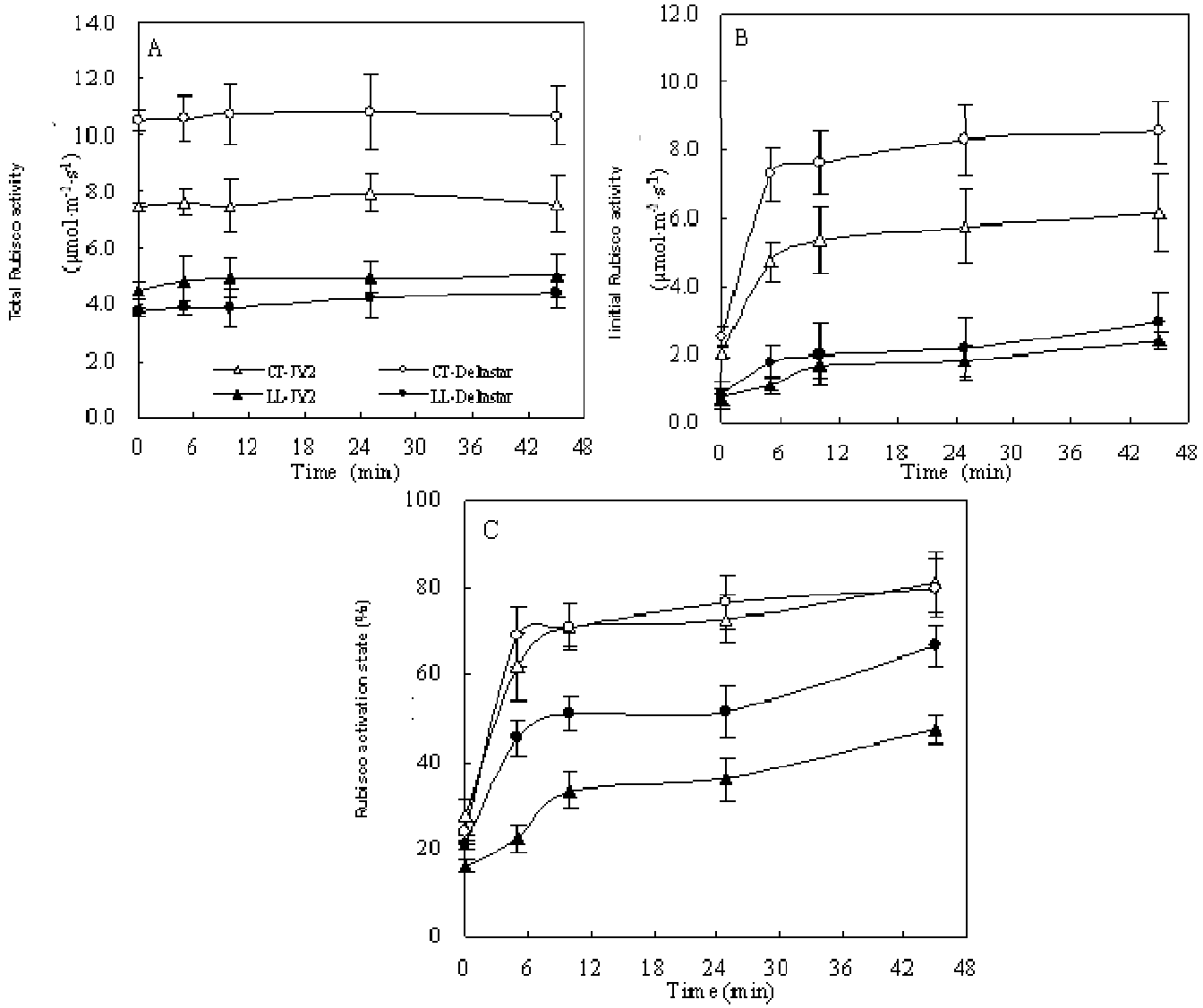

Figure 4. Changes in total Rubisco activity (A), initial Rubisco activity (B), and Rubisco activation state (C) in cucumber leaves under 500 μmol·m^{-2}·s^{-1} (CT) and 100 μmol·m^{-2}·s^{-1} (LL) irradiance respectively during photosynthetic induction period. Means ± S.D. (n = 4).

NADPH produced during the light reaction. The PSII reaction center opened and Φ_{PSII} increased very quickly. For the leaves grown under CT or low light environment, the damage of excessive energy to the reaction center could be alleviated through dissipation to heat during photosynthetic induction, showing an increase in NPQ (Figure 2B). However, there likely existed difference in both kinds of leaves on start-up speed and capacity for excessive energy dissipation. Demmig-Adams and Adams (1994) reported that the sun leaves possessed large xanthophyll cycle pools and a greater maximal zeaxanthin (antheraxanthin) contents and also displayed a greater maximal capacity in photoprotective energy dissipation in the pigment bed when compared with the leaves acclimated to very low irradiance.

Most studies have indicated that a decrease in the net photosynthetic rate under low light is a result of non-stomata restriction caused by RuBP carboxylation restriction and regeneration restriction (Pessarakli, 1997). The reduced capacity for CO_2 fixation in Calvin cycle induced a down-regulation in the PSII photochemistry. There was a feedback mechanism to inhibit the photosynthetic electron transport to match the lower demand for ATP and NADPH in the stroma of chloroplasts (Yuan and Xu, 2001). In this study, the start-up time of photosynthesis was longer in JY2 than that in Deltastar under low light. The decrease in P_N of steady state was greater in the former than in the latter when compared with their respective control. However, there were no significant differences in the length of the light reaction induction period related to chlorophyll fluorescence parameter such as Φ_{PSII} between two cucumber varieties. Thus, it was

presumed that such difference in the photosynthetic induction between varieties was mainly due to dark photosynthesis reaction under low light. Accordingly, the CO_2 assimilation in cucumber leaves of JY2 was sensitive to low light and the capacity for photosynthetic carbon fixation in the leaves of Deltastar was relatively stronger.

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Abbreviations

Chl, Chlorophyll; C_i**,** intercellular CO_2 concentration ; **CT,** the control level light; F_o**,** minimal fluorescence in dark-adapted state; F_m**,** maximum fluorescence in dark-adapted state; F_v**,** maximum variable fluorescence in dark-adapted state ($=F_m-F_o$); F_v / F_m**,** maximum quantum yield of photosystem II photochemistry; F_s**,** steady-state fluorescence under irradiance; F_m'**,** maximum fluorescence in light-adapted state; F_v'**,** maximum variable fluorescence in light-adapted state ($=F_m'-F_o'$); g_s**,** stomatal conductance; ***J,*** the linear electron transport rate; **LL,** low light**;** L_s**,** stomata limitation value; **NPQ,** non-photochemical quenching; P_N**,** net photosynthetic rate; **PPFD,** photosynthetic photon flux density; **PSII,** photosystem II; Φ_{PSII}**,** the actual photosystem II efficiency under irradiance; **Rubisco,** ribulose-1, 5-bisphosphate carboxylase/oxygenase; **RuBP,** ribulose-1,5-bisphosphate; **STP,** starting time of photosynthesis.

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