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RESEARCH ARTICLE

Photosynthetic characteristics of three varieties of *Lilium* ''Oriental Hybrids'' in the central areas of Yunnan Province, China

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Abstract To understand the ecophysiological adaptation of *Lilium* "Oriental Hybrids", which are grown for their commercial bulbs, the gas exchange, leaf N and chlorophyll content of the three varieties were investigated in the central areas of the Yunnan Province. Among the three varieties, light-saturated photosynthetic rate at ambient CO_2 (A_{max}) of Tiber was the highest, while that of Siberia was the lowest. The difference in the A_{max} was related to the carboxylation efficiency (CE), leaf mass per unit area and leaf N content per mass, which indicated that their photosynthetic capacity was influenced by the activity and/or the quantity of Rubisco. The three varieties had lower photosynthetic saturation points and photosynthetic compensation points, but the photosynthetic rates were not decreased up to 2000 μmol·m^{-2}·s^{-1} of the light intensity. This indicates that the three varieties had broad adaptability to light intensity. There were significant differences in the photosynthetic optimum temperature among the three varieties. Siberia had the highest photosynthetic optimum temperature (25.5°C–34.9°C), and is likely to grow well in warm areas. Sorbonne had the lowest photosynthetic optimum temperature (19.3°C–25.6°C), and its growth is favored in cool areas. Tiber can maintain a high photosynthetic rate within a wide range of temperature. Therefore, Tiber is the most suitable variety for the climate in the central areas of the Yunnan Province, China.

Keywords East lily (*Lilium* "Oriental Hybrids"), photosynthesis, carboxylation efficiency, leaf dry mass per area, leaf N content per mass

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1 Introduction

East lily, *Lilium* "Oriental Hybrids" (*Liliaceae, Lilium* L.), is one of the most famous flower bulbs in the world. Its cultivars are extensively used for cut flowers.

The ornamental and commercial values of cut flowers are determined by the bulb's quality. Commercially larger and heavier East lily bulbs are sold at higher prices. By improving the quality of the bulbs, they can produce higher quality cut flowers more than small bulbs. The amount of dry matter produced through photosynthesis of the lily plant and its availability to bulb scales are the most important factors for the production of large bulbs. Therefore, environmental factors such as light and temperature, which are closely related to photosynthesis, are decisive factors for the formation and dry matter accumulation of the bulbs (Guo and Zhang, 2000).

The Yunnan Province is the largest producer of East lily cut flowers in China. However, the high quality bulbs are mainly imported from Holland at high price. The cost price of cut flowers, therefore, stays high. In order to reduce the cost, researchers in the Yunnan Province have developed methods for breeding bulbs to obtain high quality cut flowers. However, they have encountered many problems with bulb culture, such as the degradation of the bulb quality, low propagation coefficient, and poor cultivation effects. As a result, most of the bulbs can not meet the production standards for high quality cut flowers. A number of research on cut flower cultivation, post-harvested treatment of bulbs, and forced cultivation have been reported (Wang and Gregg, 1992; Wang and Breen, 1987; Miller and Langhans, 1989; Heins and Pemberton, 1982). However, there have been few studies on the photosynthetic characteristics of the East lily bulb cultivation, especially the ecophysiological adaptation of East lily in the central areas of the Yunnan Province.

We investigated the gas exchange, the contents of leaf N and chlorophyll of the three varieties of the East lily in the central areas of the Yunnan Province. In addition, the effects of the photosynthetic characteristics of the East lily on the process of bulbs' breeding and the physiological and ecological environment adaptability to the cultural environment were also explored. The results provide a theoretical basis for selecting appropriate cultural environment and developing a reasonable strategy for cultivation according to the variety of East lily.

2 Materials and methods

2.1 Materials

In late March 2005, three varieties of the East lily (Siberia, Tiber, and Sorbonne) bulbs were cultivated at the Songming County, Yunnan Province, China. The altitude of the area was 1900 m. The annual precipitation was 1027.5 mm, annual mean temperature was 14°C, and annual radiative capacity was 117.1 kcal/cm^2.

These varieties, when open-cultivated, were 6–9 cm in circumference. The plants were used for the experiment.

2.2 Methods

The most important part of lily bulb cultivation is nipping off the flower buds, which is an effective way to increase the weight and volume of the bulbs. All the measurements of leaf gas exchange were done on the 15th day after this handling, and such leaf gas exchanges were measured on a clear day with a field-portable photosynthesis measuring system (PP Systems, Hertfordshire, UK). The 9th leaf from the base of the plant was chosen because it was fully developed, matured but not senesced. The measurements were taken from 3 individual plants of each species.

2.2.1 Measurement of photosynthetic light response curve

When the photosynthetic light response (A-PPFD) curve was measured, CO_2 concentration within the leaf chamber was maintained at 350 μmol·mol^{-1} and light intensities were gradually decreased from 2000 to 0 μmol·m^{-1}·s^{-1}. These produced 12 subsequent light levels (1800, 1400, 1000, 600, 400, 300, 250, 200, 150, 100, 50 and 0 μmol·m^{-2}·s^{-1}). Before each response curve was measured, the leaves were adapted to a PAR of 1000 μmol·m^{-2}·s^{-1} and CO_2 concentration of 350 μmol·mol^{-1} in order to wait for the stable photosynthetic rate. During the measurements, the minimum waiting time between steps was 5 minutes. These data were recorded when steady state was achieved. We used the software, Photosyn Assistant (V1.1, Dundee Scientific, UK) (Rothstein and Zak, 2001), to modify the curve. The software was applied to the modeling equations of Prioul and Chatier (1977) to calculate the light saturation point (LSP), the light compensation point (LCP) and the light-saturated photosynthesis at ambient CO_2 (A_{max}).

2.2.2 Measurement of photosynthetic CO_2 response curve

To produce CO_2 response curves (A–Ci), the photosynthesis values were plotted against intercellular CO_2 concentration, the light intensity was kept at 800 μmol·m^{-2}·s^{-1}, and the CO_2 concentration of the leaf chamber was gradually increased to set 15 points (1800, 1400, 1200, 1000, 800, 600, 400, 350, 300, 250, 200, 150, 100, 50 and 0 μmol·mol^{-1}). The software, Photosyn Assistant (V1.1, Dundee Scientific, UK) (Rothstein and Zak, 2001), was used to modify the curve, and was applied to the modeling equations of Mechanistic A/Ci curve analysis. These could calculate the maximum RuBP saturated rate of carboxylation (V_{cmax}) and the light-saturated rate of electron transport (J_{max}) (Stylinski et al., 2000; Takeuchi et al., 2001). The modeling of this equation was the biochemical modeling of von Caemmerer and Farquhar (1981).

2.2.3 Measurement of photosynthetic leaf temperature response curve

To produce leaf temperature response curves, photosynthesis was plotted against leaf temperature. The light intensity was kept at 800 μmol·m^{-2}·s^{-1} and the CO_2 concentration of the leaf chamber was kept at 350 μmol·mol^{-1}; the temperature of the leaf chamber was gradually increased from 14°C to 38°C. All measurements were done in the morning, when temperatures are low. The leaf temperature response curves were applied to the second order polynomial models.

2.2.4 Analysis of leaf structure and biochemistry

Fifteen leaves were collected after photosynthetic experiments and these leaves were dehydrated at 80°C for more than 48 h. The leaf mass per area (LMA, g/m^2) was thus calculated using the disc method. The leaf N content per mass was measured by the Science and Technology Department of the Yunnan Province. The leaf chlorophylls were immediately extracted by direct immersion of 20 intact discs (0.33 cm^2/disc) into the solvent N, N- dimethylformamide (DMF). The absorbance of the extracts was measured at 664.5 and 647 nm respectively with a spectrophotometer UV-2550 (Shimadzu, Japan). The leaf chlorophyll a and b content were determined using the formula of Inskeep and Bloom (1985). The calculation formulas were:

$$Chla = 12.70A_{664.5} - 2.79A_{647},$$

$$Chlb = 20.70A_{647} - 4.73A_{664.5}.$$

The measurements were done 3 times.

2.2.5 Statistical analysis

Comparisons between the varieties were made by analysis of variance (ANOVA) and LSD tests. Analyses of all the data were made with SPSS 13.0 for Windows (SPSS Inc., Chicago, USA) and the outputs of all graphs were carried out with the software SigmaPlot for windows version 10.0 (Systat Software, Inc.).

3 Results and discussion

3.1 Responses to light intensity of the three varieties of East lily

The responses to light intensity of the three varieties of East lily were similar (Table 1, Fig. 1). The light saturation point and the light compensation point were low and there was no photo-inhibition under high light intensity. These suggested that the three varieties of East lily could well adapt to the growth light intensity. Further, Tiber had the highest photosynthetic capacity among the three varieties. The photosynthetic rate determines the capacity of accumulation of dry matter, which is especially important to the bulbs of East lily.

Table 1 Photosynthetic parameters of the three varieties of *Lilium* "Oriental Hybrids"

	Siberia	Sorbonne	Tiber
A_{max}	4.497 ± 0.445^a	5.190 ± 1.207^a	6.492 ± 0.628^a
AQE	0.029 ± 0.005^a	0.034 ± 0.005^a	0.051 ± 0.012^a
LSP	373.3 ± 12.2^{ab}	274.7 ± 4.7^a	380.0 ± 5.0^b
LCP	10.7 ± 7.4^a	9.7 ± 3.5^a	4.0 ± 2.8^a
CE	0.0317 ± 0.0018^a	0.0326 ± 0.0031^a	0.0415 ± 0.0063^a
V_{cmax}	13.0 ± 0.6^a	35.0 ± 1.0^b	39.6 ± 3.3^b
J_{max}	68.0 ± 2.5^a	83.0 ± 5.9^{ab}	100.7 ± 14.1^b
N_m	0.9 ± 0^a	1.9 ± 0.1^b	1.5 ± 0^b

A_{max}: light-saturated photosynthesis at ambient CO_2 (μmol·m^{-2}·s^{-1}); AQE: apparent quantum efficiency (mol CO_2 · mol^{-1}· photons); CE: carboxylation efficiency (μmol · m^{-2}·s^{-1}); V_{cmax}: maximum rate of RuBP-mediated carboxylation (μmol · m^{-2}·s^{-1}); J_{max}: maximum rate of carboxylation limited by electron transport (μmol · m^{-2}· s^{-1}); N_m: leaf N content per mass (g · kg^{-1}); LCP: light compensation point (μmol · m^{-2}·s^{-1}); LSP: light saturation point (μmol · m^{-2}·s^{-1}). Data represent the means ± SE ($n = 3$). In the same row, a significant difference was found between different letters, a or b ($P < 0.05$).

3.2 Responses of the three varieties of East lily to carbon dioxide

Three factors determine the photosynthetic capacity: the stomatal conductance affecting the concentration of carbon dioxide in the leaf, the activity, and the regeneration capability of Rubisco (Farquhar et al., 1980; Mott, 1990). The V_{cmax} is closely related to the activity and quantity of Rubisco (Farquhar and Von Caemmerer, 1982). While

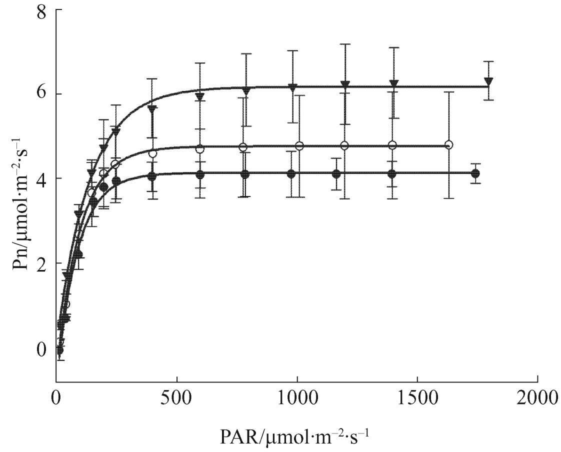

Fig. 1 Photosynthetic responses of the three varieties of *Lilium* "Oriental Hybrids" to PAR [Siberia (●-●), Tiber (▼-▼), Sorbonne(○-○)]. Each point is a mean of three measurements. The vertical bar represents ± SE.

the light-saturated rate of electron transport (J_{max}) was closely related to the regeneration capability of Rubisco.

The carboxylation efficiency (CE), the V_{cmax} and the A_{max} of the three varieties of East lily showed a similar trend (Table 1). Tiber was the highest, followed next by Sorbonne and the last was Siberia. There was no obvious difference in the A_{max} among the three varieties. The V_{cmax} of Tiber was the highest, suggesting that the activity and quantity of Rubisco was higher than the others. The J_{max} of Tiber was also significantly higher than the others, suggesting that it had the biggest potential in regenerating RuBP. Therefore, Tiber had the maximum photosynthetic capacity potential

The saturation stage of the A–Ci curve was mainly restricted by RuBP's regeneration, which was controlled by the utilization efficiency of TPU. The A–Ci curve of the three varieties of East lily did not obviously manifest the saturation stage (Fig. 2), implying that the utilization efficiency of TPU was not the main limiting factor of photosynthesis. Wei et al. (2001) found that there was no "down-regulation of photosynthesis" in the photosynthesis of the lily when it was exposed long-term to a high concentration of carbon dioxide. This may be because the lily bulbs could timely transform and store the photosynthate. Thus, the feedback suppression of photosynthate induced by the excessive photosynthate was partly eliminated in the leaves.

Leaf nitrogen content is closely related with the photosynthetic capacity, mainly because most of the leaf nitrogen is allocated to the proteins in the photosynthetic organization (Evans, 1989). Our study showed that leaf nitrogen content of Sorbonne and Tiber were higher than Siberia, but there was no obvious difference between Sorbonne and Tiber (Table 1). The change trend of leaf nitrogen was in accord with A_{max} among the different varieties.

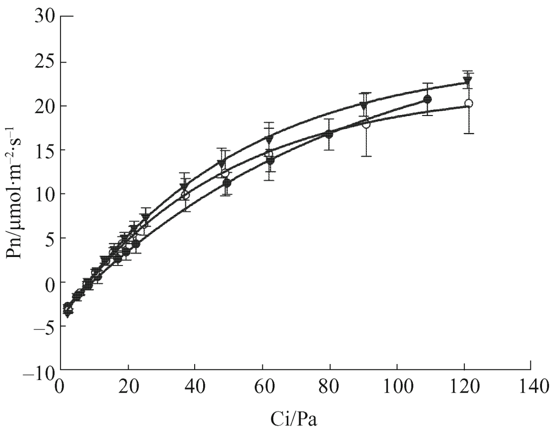

Fig. 2 Photosynthetic responses of the three varieties of *Lilium* "Oriental Hybrids" to the concentration of intercellular CO_2. Siberia: ●-●,Tiber: ▼-▼, Sorbonne: ○-○. Each point is a mean of three measurements, and vertical bar represents ± SE.

3.3 Response to leaf temperature of the three varieties of East lily

Within the optimum temperature range, the net photosynthetic rate was greater than 95% of the A_{max}. The optimum range of temperature of the three varieties, Siberia, Sorbonne and Tiber were, respectively: 25.5°C–34.9°C, 19.3°C–25.6°C and 18°C–33.1°C (Fig. 3). From 15°C to 35.1°C, the photosynthesis of Siberia, Sorbonne and Tiber had change rates of 14.8%, 29% and 7.4%, respectively. We could thus see that Siberia was a high temperature-resistant variety and its optimum temperature was the highest. Sorbonne was most sensitive to the change of temperature, and its photosynthesis showed a great change when the temperature increased or decreased. The optimum temperature range of Tiber was the widest and its photosynthetic rate was the highest.

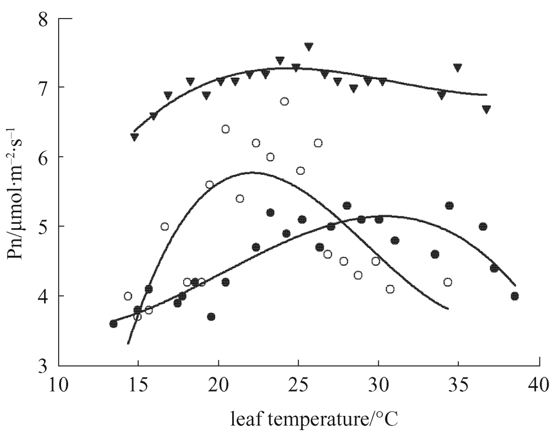

Fig. 3 Photosynthetic responses of the three varieties of *Lilium* "Oriental Hybrids" to leaf temperature. Siberia: ●-●, Tiber: ▼-▼, Sorbonne: ○-○.

3.4 Leaf characteristics of the three varieties of East lily

Leaves are the most important organs for photosynthesis. Their characteristics, for example, the content of chlorophyll and the leaf dry mass per area, directly affect the basic behavior and function of the plant. As can be found in Table 2, a significant convergence effect existed among the leaf characteristics of the three varieties of East lily, which was highest in Sorbonne, least in Siberia, with Tiber in between.

Table 2 Leaf characteristics of the three varieties of *Lilium* "Oriental Hybrids"

	Siberia	Sorbonne	Tiber
Chl a	58.7 ± 6.7^a	68.6 ± 4.3^a	64.8 ± 1.3^a
Chl b	18.8 ± 1.9^a	20.8 ± 1.19^a	20.8 ± 0.3^a
Ch a+b	77.5 ± 8.6^a	89.5 ± 5.5^a	85.7 ± 1.2^a
Chl a:b	3.11 ± 0.08^a	3.29 ± 0.03^a	3.11 ± 0.09^a
LMA	116.7 ± 3.3^a	176.7 ± 12.1^b	130.0 ± 0.0^a

Chl a: content of chlorophyll a ($\mu g \cdot g^{-1}$); Chl b: content of chlorophyll b ($\mu g \cdot g^{-1}$); Chl a+b: total content of chlorophyll ($\mu g \cdot g^{-1}$); Chl a:b: ratio of Chl a to Chl b; LMA: leaf dry mass per area($g \cdot m^{-2}$). Data represent the means ± SE ($n = 3$). In the same row, a significant difference was found between different letters, a or b($P < 0.05$).

There was no significant difference in the chlorophyll content. In other words, the light-capturing capacity of the three varieties was not significantly different. However, both the apparent quantum efficiency (AQE) and the A_{max} of Tiber were the highest (Table 1). Therefore, Tiber had the highest capacity of utilizing the light.

3.5 Correlation analysis of photosynthetic parameters of the three varieties of East lily

Fan et al. (2003) suggested that there is a related critical range between the A_{max} and LMA. If the values of LMA are too large or too small, the photosynthetic rate will decrease. LMA is important for the morphologic plasticity in plant growth adaptability (Warren and Adams, 2001; Le Roux et al., 2001; Walcroft et al., 2002; Feng et al., 2002; Zhang et al., 2003; Niienemets et al., 2003). There was a quadratic parabolic relationship between the A_{max} and LMA of the three varieties of East lily, testifying to the above viewpoint (Table 3). In a limited range, LMA was one of the most important factors affecting the photosynthesis of East lily. In other words, the East lily showed adaptive changes to the cultivation environment.

The A_{max} was significantly affected by the CE and J_{max}. It had an extremely significant correlation with the CE, suggesting that the activity and/or quantity of Rubisco were important factors affecting photosynthesis. Evans (1989) considered that Nm, which is distributed to the thylakoid membrane and stroma, is a very important factor for photosynthesis. The significant positive correlation between the Nm and V_{cmax} also confirmed this point. It

Table 3 Relationships among photosynthetic parameters of the three varieties of *Lilium* "Oriental Hybrids"

y-x	Relationship	F	R	P value
A_{max}–CE	$y = -0.198 + 15.858\ x$	14.299	0.819	0.007**
A_{max}–LMA	$y = -35.736 + 0.564x - 0.002x^2$	13.564	0.819	0.006**
V_{cmax}–N_m	$y = 0.786 + 0.024x$	6.396	0.718	0.045*
A_{max}–J_{max}	$y = 0.280 + 0.061x$	11.014	0.782	0.013*

A_{max}: light-saturated photosynthesis at ambient CO_2 (μmol · m^{-2} ·s^{-1}); V_{cmax}: maximum rate of RuBP-mediated carboxylation (μmol · m^{-2} · s^{-1}); J_{max}: maximum rate of carboxylation limited by electron transport (μmol · m^{-2} · s^{-1}); CE: the rate of carboxylation (μmol · m^{-2} · s^{-1}); N_m: leaf N concentration per mass (g · kg^{-1}); LMA: leaf dry mass per area (g · m^{-2}).
**: $P < 0.01$, *: $P < 0.05$.

implied that the leaf nitrogen affected Rubisco more than the components of photosynthetic electron transport chain. The activity and/or the number of Rubisco were the main factors affecting A_{max}.

4 Conclusions

The activity or the quantity of Rubisco being positively correlated with the leaf nitrogen content mainly caused the difference of the photosynthetic capacities among the three varieties of East lily. The significant correlation between the LMA with A_{max} within a certain range suggested that the three varieties of East lily have adapted to the cultivation environment in the central areas of the Yunnan Province morphologically.

The three varieties of East lily do not need high light intensity and have a wide adaptation to light. Tiber has the highest photosynthetic rate and carboxylation efficiency with the highest potential of photosynthesis. Siberia has the highest photosynthetic optimum temperature (25.5°C–34.9°C), and it is more likely to grow in warm areas. Sorbonne has the lowest photosynthetic optimum temperature (19.3°C–25.6°C), and its growth is favored in cool areas. Tiber can maintain a high photosynthetic rate within a wide range of temperatures. Therefore, it is a most suitable variety for the climate in the central region of the Yunnan Province.

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