

## Photosynthetic performances of five *Cypripedium* species after transplanting

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### Abstract

Photosynthesis and leaf traits of five species in genus *Cypripedium* were compared in natural habitats and transplant nursery to develop effective strategy for cultivation and conservation. Among five species, *C. guttatum* had the highest photosynthetic capacity ( $P_{Nmax}$ ) in the natural habitat and nursery, while *C. lichiangense* the lowest. The differences in  $P_{Nmax}$  among species were correlated with leaf N content (LNC) and leaf dry mass per unit area (LMA). After transplanting from natural habitats to nursery, the  $P_{Nmax}$  of *C. lichiangense* and *C. yunnanense* decreased, that of *C. guttatum* increased, while those of *C. flavum* and *C. tibeticum* remained relatively constant. The variations in LNC and biochemical efficiency would be responsible for the differences in  $P_{Nmax}$  between plants in natural habitats and in the nursery, but not the relative stomatal limitation. After transplanting, the  $F_v/F_m$  of *C. lichiangense* and *C. yunnanense* were declined. Meanwhile, the temperature ranges maintaining 90 %  $P_{Nmax}$  of *C. lichiangense* and *C. yunnanense* were narrower than those of the other three species. Thus the biochemical process in five species played a major role in the differences of  $P_{Nmax}$  after transplanting, and the widespread species had higher photosynthetic adaptability than the narrow-spread species.

*Additional key words:* alpine orchid; chlorophyll fluorescence; cultivation; leaf trait; photosynthesis.

### Introduction

The genus *Cypripedium*, known as lady's slipper orchid, contains 49 species distributed in North America, East Asia, and Europe. In the Hengduan Mountains of Southwestern China, these orchids only occur in alpine grasslands or under the scrub and forest at altitude of 2 700–3 700 m (Cribb 1997). *Cypripedium* species often have very specific and limited conditions for flowering, seed germination, and offspring production (Kull 1998, Weng *et al.* 2002). As a result, large-scale cultivation under artificial conditions is necessary for their conservation and for continued use in the ornamental trade. However, successful cultivation and conservation of wild species requires knowledge of their requirements for optimal growth (Cui *et al.* 2004). Photosynthesis is widely used as a tool for indicating environmental stress and selection of growth conditions suitable for different species (Weng

and Ueng 1997, Vats *et al.* 2002). The capacity of plants to grow and develop in regimes differing from their original habitats depends on their abilities for photosynthetic acclimation to changing environment (Percy 1977). To date, however, there are few studies on domestication and cultivation of *Cypripedium* (Kull 1998, Zhang *et al.* 2005). Indeed, although *Cypripedium* species have been cultivated for centuries, and seedling micro-propagation has been successful for several members of genus *Cypripedium* (Cribb 1997, Shimura and Koda 2004), cultivation is still difficult because the optimal growing condition remains unclear.

After transplanting from natural habitat to nursery, plants would be exposed to uncomfortable environments (Weng and Ueng 1997). The growth and development of plants in the changing environments depend on physio-

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**Abbreviations:** AQE, apparent quantum efficiency;  $C_i$ , intercellular  $CO_2$  concentration; Chl, chlorophyll; ETR, electron transport rate;  $F_v/F_m$ , maximal quantum efficiency of photosystem 2;  $I_c$ , compensation irradiance;  $I_s$ , saturation irradiance;  $J_{max}$ , photon saturated rate of electron transport; LMA, leaf dry mass per unit area; LNC, leaf nitrogen content;  $P_N$ , net photosynthetic rate;  $P_{Nmax}$ , photon saturated  $P_N$ ; PPFD, photosynthetic photon flux density; PS, photosystem; RSL, relative stomatal limitation;  $T_l$ , leaf temperature;  $T_{opt}$ , optimum temperature for photosynthesis;  $V_{cmax}$ , maximum carboxylation rate by ribulose-1,5-bisphosphate carboxylase/oxygenase;  $\Gamma$ ,  $CO_2$  compensation concentration;  $\Delta F/F_m'$ , quantum yield of photosystem 2.

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logical tolerance and genetic differentiation (Cordell *et al.* 1998). Widespread species show often higher phenotypic plasticity than endemic species (Maliakal-Witt *et al.* 2005). Phenotypic plasticity is important for the success of plant over a variety of contrasting habitats (Tsialtas *et al.* 2004). The photosynthetic traits would account for their abilities to succeed in changing environments (Gulias *et al.* 2002). The eco-physiological trait might be related to the ability of a species to cope with environmental stress. Does this imply that widespread species can be introduced and domesticated easier than

narrow spread species?

Five species with different distribution range were transplanted from three wild populations to nursery at altitude of 3 240 m. After 1-year physiological adaptation, the photosynthetic performances and leaf traits of transplanted plants were compared with their counterparts in the natural habitats. The objective was to assess the capacity for photosynthetic adaptation of different species to the changing environments and test the hypothesis that wide-spread species could be introduced and domesticated easier than narrow-spread species.

## Materials and methods

**Plants and sites:** Five species of genus *Cypripedium* were investigated in present study (for ecological and biological traits see Table 1). 50–60 seedlings (dormant) of *C. flavum* P.F. Hunt *et* Summerh and *C. yunnanense* Franch. were collected from Tianshengqiao (alt. 3 450 m, 99°50.10'E, 27°47.76'N) in March 2003, *C. lichiangense* S.C. Chen *et* Cribb from Geza (alt. 2 960 m, 99°43.09'E, 28°11.40'N), while *C. guttatum* Sw. and *C. tibeticum* King *ex* Rolfe were from Napahai (alt. 3 360 m, 99°33.46'E, 27°55.28'N). The seedlings in the nursery (alt. 3 240 m, 99°38.80'E, 27°46.10'N) were shaded by nylon netting to give 40–50 % of full sunlight, and were watered regularly during dry period. 15 seedlings of corresponding size of each species were marked for measurements at their natural habitats.

The studies were done at three natural habitats and a transplant nursery in the Hengduan Mountains in June 2004 (flowering period). Four sites have similar climatic conditions because the altitudinal and lateral distances separating them are both small. In this area, annual mean temperature was 5.4 °C and annual precipitation was 624.8 mm (Weng *et al.* 2002).

**Chlorophyll (Chl) fluorescence** was measured with a FMS-2 pulse modulated fluorometer (Hansatech, Norfolk, UK) in the morning. After the minimal fluorescence ( $F_0$ ) was determined by a weak modulated radiation, a 0.8 s saturating irradiance of 8 000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  was used on dark-adapted leaf (30 min) to determine the maximal fluorescence ( $F_m$ ). Then the leaf was irradiated by an actinic radiation of 600  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . After 5 min, the steady-state fluorescence ( $F_s$ ) was recorded and a second 0.8 s saturating irradiance of 8 000  $\mu\text{mol m}^{-2}\text{s}^{-1}$  was given to determine the maximal fluorescence ( $F_m'$ ) of the light-adapted leaf. Ten leaves were measured for each species. The following parameters were calculated (Genty *et al.* 1989): (1)  $F_v/F_m = (F_m - F_0)/F_m$ ; (2)  $\Delta F/F_m' = 1 - F_s/F_m'$ ; (3)  $\text{ETR} = \Delta F/F_m' \times \text{PPFD} \times 0.85 \times 0.5$ , where 0.5 is a factor assuming an equal distribution of absorbed photons between photosystem (PS) 1 and PS2, and leaf absorbance was taken as 0.85.

**Photosynthetic responses** to photosynthetic photon fluence density (PPFD) were measured on fully expanded leaves using a CIRAS-1 infrared gas analyzer (PP Systems, Hertfordshire, UK) under 20 °C and 350  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentration. After the initial measurement at 0  $\mu\text{mol m}^{-2}\text{s}^{-1}$ , PPFD was increased to produce 10 subsequent levels at which net photosynthetic rate ( $P_N$ ) was recorded. Three plants were measured for each species at any site. Data were fit by a non-rectangular hyperbola (Prioul and Chartier 1977). Using this function,  $P_{N\text{max}}$ , AQE, compensation irradiance ( $I_c$ ), and saturation irradiance ( $I_s$ ) were estimated by *Photosyn Assistant* software (Dundee Scientific, Scotland, UK).

Following  $P_N$ -PPFD curves,  $P_N$ - $C_i$  curves were generated to investigate photosynthetic characteristics. For each species, three recently matured leaves were placed in the leaf chamber at a  $\text{CO}_2$  concentration of 350  $\mu\text{mol mol}^{-1}$ , 20 °C, and 600  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD. Following a 15 min acclimation period,  $P_N$  was recorded, then the  $\text{CO}_2$  concentration was reduced to 50  $\mu\text{mol mol}^{-1}$  and another reading was taken. A gradual increase in ambient  $\text{CO}_2$  to a final concentration of 2 000  $\mu\text{mol mol}^{-1}$  in 10 increments occurred with a reading taken at each increment following a 3-min acclimation period. Using  $P_N$ - $C_i$  curves,  $V_{c\text{max}}$  and  $J_{\text{max}}$  were calculated by *Photosyn Assistant* software that applied the biochemical model described by Caemmerer and Farquhar (1981). RSL of photosynthesis, an estimate of proportion of the reduction in photosynthesis attributable to  $\text{CO}_2$  diffusion between atmosphere and site of carboxylation, was calculated from  $P_N$ - $C_i$  curves by the method of Farquhar and Sharkey (1982).

The dependence of photosynthesis on temperature was examined with fully expanded leaves using an infrared gas analyzer in the morning to avoid high temperature at midday. Measurements were made between 10 and 35 °C at  $\text{CO}_2$  concentration of 350  $\mu\text{mol mol}^{-1}$  and PPFD of 600  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . A second-order polynomial equation was used to fit optimum temperature for photosynthesis ( $T_{\text{opt}}$ ).

**Analysis of leaf trait:** Following photosynthetic responses, the leaves were harvested from the sample plants. In the laboratory, leaf areas were measured by leaf area meter (*LI-3000A*, Lincoln, NE, USA). Dry mass was determined after drying for 48 h at 70 °C. Then, leaf N content was analyzed using an N analyzer (*Leco FP-428*,

Michigan, USA). LMA was calculated as dry mass per unit leaf area [ $\text{g m}^{-2}$ ]. Chl was extracted with N,N-dimethylformamide, analyzed with a spectrophotometer (*UV-2550*, *Shimadzu*, Kyoto, Japan), and calculated using the method of Inskeep and Bloom (1985). Three repetitions were made for each species.

Table 1. The experimental sites and biological traits of five *Cypripedium* species. *C. guttatum* is a widespread species in the Northern Hemisphere, *C. flavum* is an endemic congener in China, while *C. lichiangense*, *C. yunnanense*, and *C. tibeticum* are distributed in Himalaya.

	<i>flavum</i>	<i>guttatum</i>	<i>lichiangense</i>	<i>tibeticum</i>	<i>yunnanense</i>
Distribution	China	Europe, Asia, North America	W. China, NE. Burma	Sikkim, Bhutan, W. China	Yunnan in China
Altitude [m]	1 800–3 700	1 000–4 100	2 600–3 500	2 300–4 200	2 700–3 800
Height [cm]	17–60	10–38	15–20	13–35	20–37
Number of leaves	6–7	2	2	6	5–6
Leaf length [cm]	9.0–17.5	4.5–12.5	8.5–19.0	7.0–15.0	6.0–14.0
Leaf width [cm]	4.2–12.0	2.5–3.5	7.0–16.0	3.3–7.2	1.0–4.1
Flowering period	Jun–Jul	May–Jul	Jun	May–Jun	Jun–Jul
Fruiting period	Jul–Oct	Jun–Sep	Jul–Oct	Jul–Oct	Jul–Oct
Natural habitat	Tianshengqiao	Napahai	Geza	Napahai	Tianshengqiao

**Statistical analysis** was performed using *SPSS* version 10.0 (*SPSS*, Chicago, USA). Differences in leaf morphological and physiological variables among species were determined by using one-way ANOVA and LSD

multiple comparisons test, differences between native habitat and nursery determined by *t*-test. The relationships between photosynthetic parameters and leaf traits were assessed using linear regression analysis.

## Results

**Chl fluorescence:** After transplanting, the values in  $F_v/F_m$  of *C. flavum*, *C. guttatum*, and *C. tibeticum* did not change, but those of *C. lichiangense* and *C. yunnanense* decreased significantly (Fig. 1). This indicated that *C. lichiangense* and *C. yunnanense* were exposed to stress in the nursery. The values in  $\Delta F/F_m'$  and ETR of *C. lichiangense* and *C. yunnanense* in natural habitats were higher than those in the nursery, but in other three species remained relatively constant (Fig. 1).

**Photosynthetic responses to  $T_i$ , PPFD, and  $C_i$ :** In natural habitats, *C. guttatum* and *C. lichiangense* had higher  $T_{opt}$  than those of other three species ( $p=0.023$ ), but there were no significant differences in the five species in the nursery ( $p=0.884$ ). After transplanting, the  $T_{opt}$  of five species did not change obviously.  $P_N$  remained in excess of 90 % of maximum between 12 and 26 °C for *C. flavum*, between 13 and 28 °C for *C. guttatum*, between 15 and 27 °C for *C. lichiangense*, between 12 and 27 °C for *C. tibeticum*, and between 12 and 23 °C for *C. yunnanense*. Both *C. lichiangense* and *C. yunnanense* maintained relatively high  $P_N$  at narrower temperature range than the other three species (Fig. 2).

In natural habitats,  $P_N$  of five species exhibited similar response to PPFD (Fig. 3). Although *C. lichiangense* had the lowest  $I_s$  among five species, there were no

significant differences in  $I_s$  ( $p=0.057$ ) and  $I_c$  ( $p=0.447$ ). They became photon saturated between 505.3 and 621.7  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After transplanting, the  $I_c$  and  $I_s$  of *C. flavum*, *C. guttatum*, *C. tibeticum*, and *C. yunnanense* did not change obviously, but the  $I_c$  of *C. lichiangense* increased ( $p=0.047$ ) and  $I_s$  decreased ( $p=0.049$ ). Among the five species, *C. guttatum* had the highest  $P_{Nmax}$  and AQE, while *C. lichiangense* had the lowest values (Table 2). After transplanting, the  $P_{Nmax}$  of *C. guttatum* increased, of *C. lichiangense* and *C. yunnanense* decreased, while of *C. flavum* and *C. tibeticum* remained relatively constant.

In natural habitats, the  $P_N-C_i$  response curve of *C. lichiangense* was different from those of the other four species (Fig. 4). *C. lichiangense* exhibited a higher  $\text{CO}_2$  compensation concentration ( $\Gamma$ ) than the other species. After transplanting,  $\Gamma$  of *C. lichiangense* and *C. yunnanense* increased, while that of the other three species did not change obviously (Table 2). Among the five species, *C. guttatum* had the highest  $V_{cmax}$  and  $J_{max}$  than the other four species, but there were no significant differences in RSL among species or experimental sites. The  $P_{Nmax}$  was related to  $J_{max}$  and  $V_{cmax}$ , respectively, not but to RSL (Table 3).

**Leaf traits of five species:** Chl content of *C. tibeticum*

was lower than those of the other four species both in natural habitats ( $p=0.012$ ) and nursery ( $p=0.047$ ), but there were no differences for five species between natural habitats and nursery. Chl contents of five *Cypripedium* species were not closely correlated with  $P_{Nmax}$  (Table 3). After transplanting, LNC of *C. lichiangense* and *C. yunnanense* decreased, but that of other three species

## Discussion

**Photosynthetic capacities of the five species:**  $P_{Nmax}$  differed significantly across species, varying from 4.0 to 10.5  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . Both in natural habitat and nursery, *C. guttatum* exhibited the highest  $P_{Nmax}$  among the five species, while *C. lichiangense* the lowest one. The differences in  $P_{Nmax}$  reflected differences in leaf physiology, anatomy, and biochemistry (Durand and Goldstein 2001).

There was a strong positive correlation between  $P_{Nmax}$  and LNC because of the large proportion of leaf N present in the photosynthetic apparatus (Poorter and Evans

1998). We confirmed this result for the five *Cypripedium* species. LNC was significantly related to  $P_{Nmax}$ ,  $J_{max}$ , and  $V_{cmax}$  (Table 3). Higher leaf N content would be a critical determinant of higher  $P_{Nmax}$  of *C. guttatum*.

Photosynthetic capacity depended on either photosynthetic biochemistry or leaf anatomy (Reich *et al.* 1998). However, the importance of leaf structural parameters has been argued. Thick leaves not only had higher LNC and ribulose-1,5-bisphosphate carboxylase/oxygenase content, but also larger resistance to  $\text{CO}_2$  diffusion. Although many studies showed that high LMA was often associated with higher LNC and  $P_{Nmax}$  (Poorter and Evans 1998), this was not true in the five *Cypripedium* species. Similar results were observed by other researchers (Mediavilla *et al.* 2001, Niinemets *et al.* 2002). The reason was that thick leaves with high LMA had a low allocation of N to photosynthetic machinery and small photosynthetic N-use efficiency, thereby lower  $P_N$  (Mediavilla *et al.* 2001). Among the five species, *C. lichiangense* had the highest LMA, not LNC, while *C. guttatum* had the lowest LMA with higher LNC. This discrepancy would be largely due to difference in N absorption ability of plants (Osone and Tateno 2005).

**Physiological adaptation of the five species:** No uniform trend was observed in variations of  $P_{Nmax}$  after transplanting. In *C. guttatum*,  $P_{Nmax}$  increased by 8.4 % in the nursery compared to that at its natural habitat.  $P_{Nmax}$  of *C. lichiangense* and *C. yunnanense* decreased by 33.2 and 17.8 %, respectively, but there were no significant differences in  $P_{Nmax}$  of *C. flavum* and *C. tibeticum* between in the natural habitats and nursery. The variations in  $P_{Nmax}$  of the five species after transplanting would be linked to the changes of AQE,  $V_{cmax}$ ,  $J_{max}$ , and LNC, not to the changes of LMA, Chl, and RSL. Obviously, photosynthesis would be limited mainly by biochemical process after transplanting, not stomatal limitation. This also could be confirmed by the increase of  $\Gamma$  in *C. yunnanense* and *C. lichiangense* after transplanting. The decrease in  $P_{Nmax}$  of *C. yunnanense* and *C. lichiangense* after transplanting would be due to the decrease in N absorption ability (Osone and Tateno 2005), while the increase in  $P_{Nmax}$  of *C. flavum*, *C. guttatum*, and *C. tibeticum* would be attributable to lack of strong environmental limitation in the nursery where conditions remained constant (Cordell *et al.* 1998).

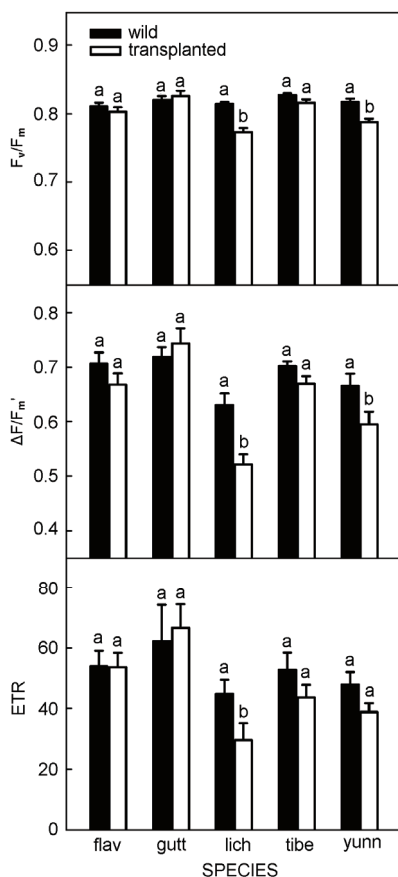


Fig. 1. Comparisons of maximal quantum efficiency of photosystem 2, PS2 ( $F_v/F_m$ ), quantum yield of PS2 ( $\Delta F/F_m'$ ), and electron transport rate (ETR) of *C. flavum* (flav), *C. guttatum* (gutt), *C. lichiangense* (lich), *C. tibeticum* (tibe), and *C. yunnanense* (yunn) in the natural habitat (open columns) and nursery (full columns). Vertical bars indicate standard errors of mean for 10 measurements. Different letters represent significant differences ( $t$ -test,  $p<0.05$ ).

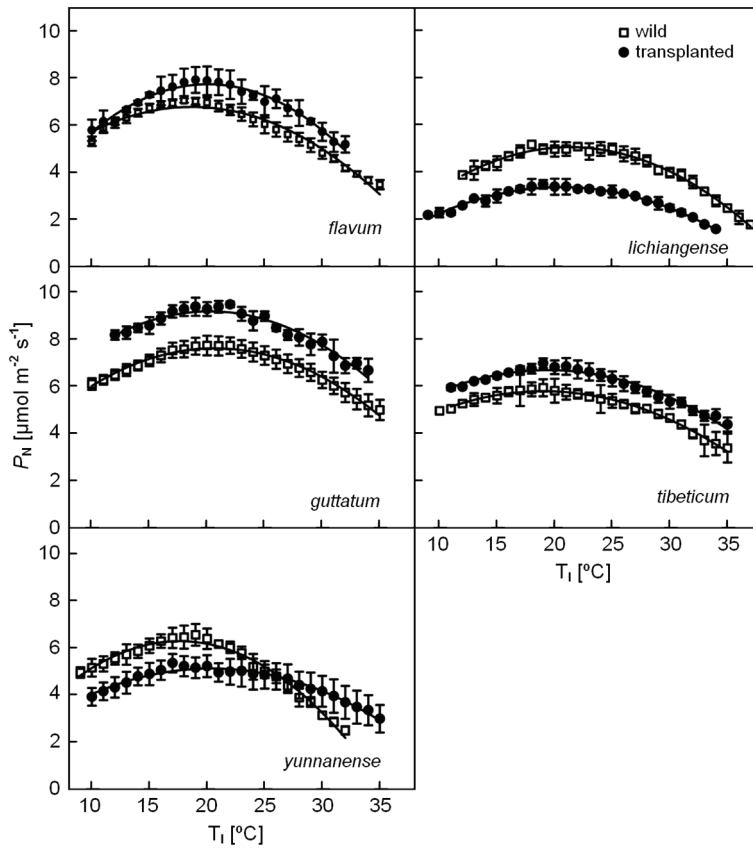


Fig. 2. Effects of leaf temperature on the photosynthetic rates of *C. flavum*, *C. guttatum*, *C. lichiangense*, *C. tibeticum*, and *C. yunnanense* in the natural habitat (□) and nursery (●). Vertical bars indicate standard errors of mean for 3 measurements.

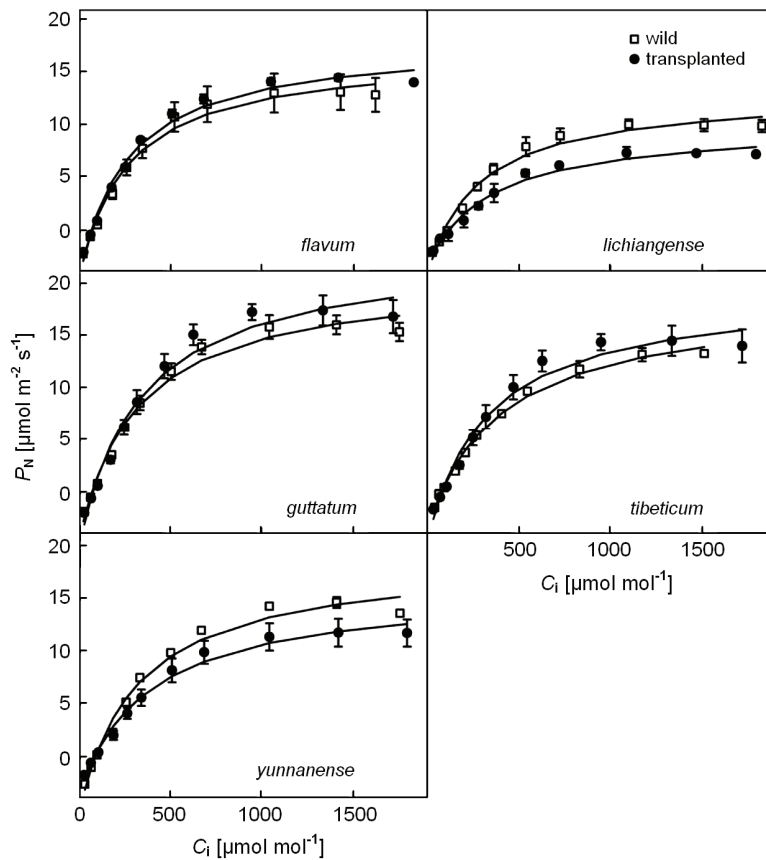


Fig. 3. Photosynthetic responses to photosynthetic photon flux density (PPFD) of *C. flavum*, *C. guttatum*, *C. lichiangense*, *C. tibeticum*, and *C. yunnanense* in the natural habitat (□) and nursery (●). Vertical bars indicate standard errors of mean for 3 measurements.

Table 2. Comparisons of photosynthetic parameters and leaf traits of five *Cypripedium* species in natural habitat and nursery. Different letters within same row indicate mean values statistically different at  $p < 0.05$  as determined by LSD test. ns ( $p > 0.05$ ), \* ( $p < 0.05$ ), and \*\* ( $p < 0.01$ ) represent the differences between natural habitat and transplant nursery as determined by *t*-test.

		Species				
		<i>flavum</i>	<i>guttatum</i>	<i>lichiangense</i>	<i>tibeticum</i>	<i>yunnanense</i>
$P_{Nmax}$	wild	8.95±0.51a	9.69±0.20a	6.03±0.27b	6.76±0.40c	7.63±0.37c
	trans	9.23±0.37a	10.50±0.22b	4.03±0.26c	8.02±0.43d	6.27±0.23e
	<i>p</i>	ns	*	**	ns	*
AQE	wild	0.045±0.001ac	0.067±0.012ab	0.036±0.003c	0.047±0.009ac	0.040±0.001c
	trans	0.042±0.004ad	0.064±0.004b	0.015±0.001c	0.049±0.007a	0.032±0.002d
	<i>p</i>	ns	ns	**	ns	*
$V_{cmax}$	wild	22.50±2.69a	21.90±1.46a	17.31±0.51a	18.67±0.85a	20.63±1.57a
	trans	22.85±0.94a	27.27±1.04a	12.67±1.07b	23.40±2.56a	16.53±1.02b
	<i>p</i>	ns	*	*	ns	*
$J_{max}$	wild	81.83±9.07cd	105.17±6.95a	60.10±1.94bc	69.53±5.39de	84.73±8.60ac
	trans	87.71±3.80a	110.67±3.48b	44.77±1.33c	88.53±10.5a	69.13±4.96d
	<i>p</i>	ns	ns	**	ns	ns
$\Gamma$	wild	77.90±2.04ab	74.50±10.80a	107.53±1.94b	66.53±18.2a	70.13±2.48a
	trans	66.01±5.78a	79.47±2.36ac	136.70±12.7b	73.87±4.75a	96.97±6.42c
	<i>p</i>	ns	ns	*	ns	*
RSL	wild	12.18±1.86a	13.96±1.27a	11.61±0.54a	25.46±3.04b	14.05±0.75a
	trans	12.34±0.21a	14.11±0.96a	16.92±3.78a	16.76±1.44a	15.76±2.01a
	<i>p</i>	ns	ns	ns	ns	ns
LMA	wild	65.30±2.46a	55.99±2.36b	69.88±1.03ac	63.20±3.58ab	61.33±2.20b
	trans	64.77±3.5ab	59.64±3.47a	69.64±0.73b	60.69±0.06b	58.36±2.10a
	<i>p</i>	ns	ns	ns	ns	ns
Chl	wild	1.76±0.02a	1.79±0.08a	1.93±0.05a	1.47±0.06b	1.78±0.11a
	trans	1.79±0.06a	1.80±0.09a	1.91±0.04b	1.60±0.05b	1.83±0.05a
	<i>p</i>	ns	ns	ns	ns	ns
LNC	wild	0.711±0.057a	0.707±0.043a	0.657±0.009ac	0.560±0.026bc	0.630±0.026ac
	trans	0.760±0.050a	0.850±0.015b	0.540±0.015c	0.623±0.009d	0.533±0.009c
	<i>p</i>	ns	ns	**	ns	*

Table 3. Regression analysis of factorial correlation for five *Cypripedium* species.  $\Delta P_{Nmax}$  means the  $P_{Nmax}$  values of transplanted plants minus those of wild plants. Other parameters were calculated by the same method.

Y-X	a (±SE)	b (±SE)	<i>F</i>	$r^2$	<i>p</i>
$P_{Nmax}$ -AQE	3.768 (0.660)	90.586 (14.176)	40.831	0.593	0.000
$P_{Nmax}$ - $V_{cmax}$	0.523 (0.962)	0.353 (0.046)	58.620	0.677	0.000
$P_{Nmax}$ - $J_{max}$	1.232 (0.722)	0.081 (0.009)	86.099	0.755	0.000
$P_{Nmax}$ -LNC	-1.271 (1.513)	13.676 (2.273)	36.209	0.564	0.000
$P_{Nmax}$ -RSL	9.575 (1.199)	-0.121 (0.075)	2.633	0.086	0.116
$P_{Nmax}$ - $\Gamma$	12.279 (0.964)	-0.054 (0.011)	24.290	0.465	0.000
$P_{Nmax}$ -Chl	11.368 (3.992)	-2.067 (2.249)	0.844	0.029	0.366
$P_{Nmax}$ -LMA	14.497 (3.665)	-0.108 (0.058)	3.455	0.110	0.074
$V_{cmax}$ -LNC	1.053 (3.850)	29.403 (5.784)	25.844	0.480	0.000
$\Delta P_{Nmax}$ - $\Delta$ AQE	0.768 (0.382)	147.038 (37.187)	15.634	0.839	0.029
$\Delta P_{Nmax}$ - $\Delta V_{cmax}$	-0.300 (0.202)	0.287 (0.048)	36.434	0.924	0.009
$\Delta P_{Nmax}$ - $\Delta J_{max}$	-0.191 (0.195)	0.090 (0.015)	38.771	0.928	0.008
$\Delta P_{Nmax}$ - $\Delta$ RSL	-0.264 (0.413)	-0.226 (0.089)	6.373	0.680	0.086
$\Delta P_{Nmax}$ - $\Delta$ LMA	-0.133 (0.724)	0.149 (0.302)	0.194	0.061	0.690
$\Delta P_{Nmax}$ - $\Delta$ LNC	-0.298 (0.273)	11.761 (2.731)	18.547	0.861	0.023
$\Delta P_{Nmax}$ - $\Delta$ Chl	-0.527 (0.395)	7.731 (2.706)	8.162	0.731	0.065

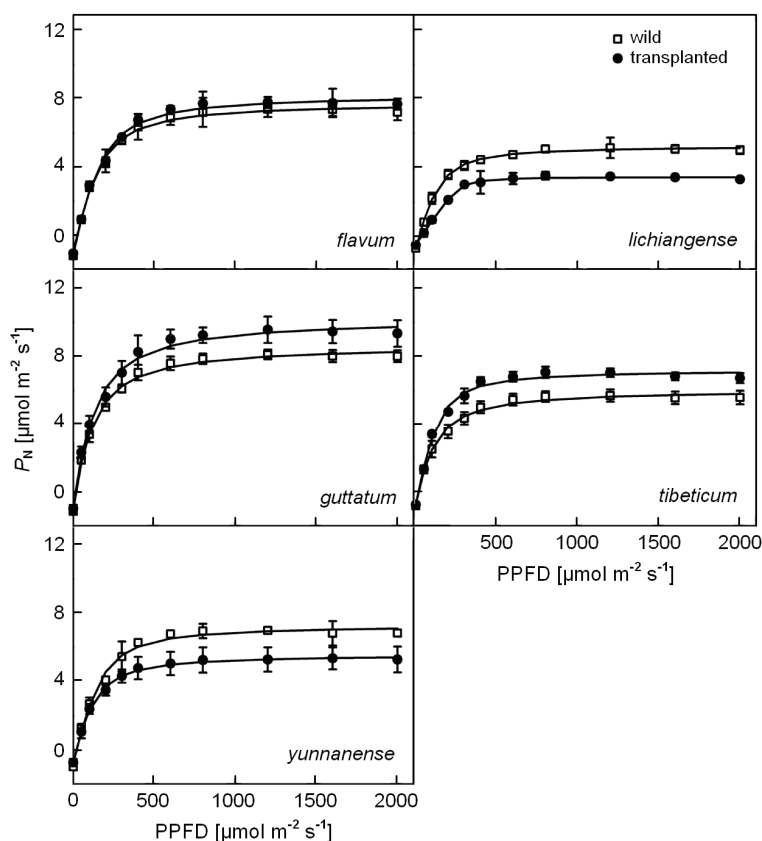


Fig. 4. Photosynthetic CO<sub>2</sub> responses of *C. flavum*, *C. guttatum*, *C. lichiangense*, *C. tibeticum*, and *C. yunnanense* in the natural habitat (□) and nursery (●). Vertical bars indicate standard errors of mean for 3 measurements.

The variation in photosynthesis would reflect the physiological adjustment to the changing environments. Since there was no significant difference in air temperature among study sites, the  $T_{opt}$  of five species in the nursery were similar to those at their natural habitats, but the temperature ranges maintaining 90 %  $P_{Nmax}$  of *C. lichiangense* and *C. yunnanense* were narrower than those of the other three species. Except for *C. lichiangense*, other four species had similar  $I_s$  and  $I_c$  both in natural habitats and nursery. The  $I_c$  of *C. lichiangense* increased after transplanting,  $I_s$  decreased. This indicated that the ability of *C. lichiangense* to utilize photon energy was decreased. Furthermore, the values of  $F_v/F_m$ ,  $\Delta F/F_m'$ , and ETR in *C. lichiangense* and *C. yunnanense* in the nursery were lower than those in the natural habitats. *C. lichiangense* and *C. yunnanense* were considerably exposed to stress, thereby lower photochemical efficiency and  $P_N$ .

Baskauf and Eichmeir (1994) suggested that photosynthetic differences could not account for contrasting geographic distributions of the endemic species and their

widespread congeners, but several studies showed that endemic species had lower photosynthetic capacity than widespread congeners (Gulías *et al.* 2003, Cui *et al.* 2004). The greater phenotypic plasticity (e.g.  $P_N$ ) would be of value for survival and success of plants (Gulías *et al.* 2002). In the present study, three widespread species not only had higher  $P_{Nmax}$  than the two narrow-spread species, but also broader physiological adaptability to the changing environments. From physiological view, the possibility of cultivating three wide-spread species under artificial condition was higher than that of the two narrow-spread species.

In conclusion, the wide-spread species of the genus *Cypripedium* had higher  $P_{Nmax}$  after transplanting than the narrow-spread species. This difference would be linked to the differences in physiological adaptability and LNC, not but to stomatal limitation. The results support the hypothesis that wide-spread species have relatively broad physiological adaptability, and could be introduced and domesticated easier than narrow-spread species.

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