Comparative photosynthesis characteristics of *Calycanthus chinensis* and *Chimonanthus praecox*

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Abstract

Calycanthus chinensis is an endangered plant of the national second-grade protection of China restricted in a small area in Zhejiang Province. We studied parameters of photosynthesis, chlorophyll (Chl) contents, and Chl fluorescence (minimum fluorescence, F_0, maximum fluorescence, F_m, variable fluorescence, F_v, and F_v/F_m) of *C. chinensis* and *Chimonanthus praecox*. *C. chinensis* had lower compensation irradiance but higher saturation irradiance than *C. praecox*. Hence *C. chinensis* has more advantage in obtaining and utilizing photon energy and higher Chl content, and is more adaptive to higher temperature and propitious to thermal dissipation than *C. praecox*. In addition, *C. chinensis* produces abundant, well-preserved seed with a higher germination rate and a wider adaptability to temperature than *C. praecox*. Thus *C. chinensis* is prone to survival and viability, and gets rid of the endangered plant species of the national second-grade protection of China.

Additional key words: biodiversity; chlorophyll content and fluorescence; endangered plant; irradiance; net photosynthetic rate; species differences; temperature; transpiration.

Introduction

Calycanthus chinensis is the only representative species in China in the genus. It is endemic to China and dates to Tertiary. Because of its unique taxonomic status, narrow distribution area, and small population, it is an endangered plant of the national second-grade protection of China (Zhang and Mao 1992). It is restricted to a small area of Lin'an City (119.72E, 30.23N) and on a hill of Tiantai Country (121.03E, 29.15N) in Zhejiang Province. It is very similar to *Calycanthus floridus* L., a common ornamental, which is the only species of this genus that is distributed in North America. This phenomenon might be due to the continental drift (Xiang *et al.* 1998). The distribution of the two *Calycanthus* species can be an important evidence for explaining the theory of the plate tectonics. Hitherto the classification, ecological character, karyotype analysis (Li 1986, Liu *et al*. 1996), propagation and cultivation, and genetic diversity (Zhou and Ye 2002) of *C. chinensis* had been studied, however, little information is known about its photosynthetic characteristics. *Chimonanthus praecox*, as one of the most wide-planted floricultures in China, is distributed in subtropical, monsoon, and humid-climate regions. We compared the photosynthetic characters of *C. chinensis* with those of *C. praecox* in order to find the differences between their ecophysiological characteristics, and the theoretical basis for the distribution and culture of *C. chinensis*.

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Abbreviations: Chl – chlorophyll; CI – compensation irradiance; E – transpiration rate; F_0 – minimum fluorescence; F_m – maximum fluorescence; F_v – variable fluorescence; PAR – photosynthetically active radiation; P_N – net photosynthetic rate; P_S – P_N at saturation irradiance; PS – photosystem; Q_A – redox state of the quinone acceptor A in PS2; q_{NP} – non-photochemical quenching; q_P – photochemical quenching; RC – reaction centre; SI – saturation irradiance; T_{leaf} – leaf temperature; Φ_{PS2} – effective quantum yield of PS2.
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Materials and methods

Plants: *C. chinensis* was introduced from the Tianmu mountains of Lin'an City and planted in Zhejiang Normal University campus together with *C. praecox*. The two plant species grew well in the natural environment.

Chlorophyll (Chl) contents: Chls were extracted by grinding leaves in 80 % acetone. Absorbance of the resulting extracts was measured at two wavelengths: 663.6 and 646.6 nm. Chl contents in these extracts were determined from the equations given by Porra (2002).

Gas exchange and related parameters: The measurements of net photosynthetic rate (P_N), transpiration rate (E), leaf temperature (T_{leaf}), and photosynthetically active radiation (PAR) were carried out using a *LCA-4* portable photosynthesis system (*ADC Bio Scientific,* Hoddesdon, UK). The parameters were measured on uppermost, fully expanded leaves from 07:00 to 17:30 in bright sunlight on a clear, cloudless day. Five replications were done for each plant at each time.

Chl fluorescence measurements were taken using *PAM-210* chlorophyll fluorometer (*Walz*, Effeltrich, Germany). Before fluorescence measurement, the plants were dark adapted for at least 30 min. The Chl fluorescence and maximum PS2 efficiency (F_v/F_m) were measured under weak red radiation. Minimum fluorescence (F_0) was determined with the measuring radiation (0.04 μmol m^{-2} s^{-1}). Maximum fluorescence of dark-adapted leaf (F_m) was measured under a subsequent saturating pulse of red radiation. After F_m determination, the leaves were exposed to "actinic light" and a sequence of saturation pulses (4 ms per pulse) was provided in order to realize the fluorescence induction curves with quenching analysis. After every saturation pulse, the values of the effective quantum yield of PS2 (Φ_{PS2}), the photochemical quenching (q_P), and the non-photochemical quenching (q_{NP}) were calculated using the *PAM-210* fluorometer software. Measurements were carried out in selected attached leaves. The wavelengths of excitation (650 nm) and detection (>700 nm) were embedded in devices of *PAM-210*.

Data analysis: Each experiment was conducted as a randomized complete block design and was repeated at least three times. The statistical significance of the Chl contents and parameters of Chl fluorescence between the two species were compared using *t*-test with *SAS* version *9.0* (*SAS Institute*, Cary, NC, USA).

Results

Photosynthesis: The measurements on *C. chinensis* and *C. praecox* were made in the same day. The average P_N of leaves of the two species (Fig. 1*A*) increased rapidly from 07:00 to a maximum at approximately 08:30. This was followed by a progressive decrease, which was smaller and delayed in *C. chinensis*, especially during the period from 11:30 to 13:00. After that, both P_N and E in *C. praecox* were distinctly lower (Fig. 1*B*) than those in *C. chinensis*. There was no significant difference in the maximum P_N between the two species. However, the decrease in P_N from the early morning maximum to midday of *C. praecox* was significant compared with *C. chinensis*.

Before the solar radiation reached 1 500 μmol m^{-2} s^{-1}, P_N of the two species increased accordingly with the increasing PAR (Fig. 2). SI (saturation irradiance) in *C. chinensis* was a little higher than that in *C. praecox*. After that there was a slight decrease in *C. chinensis* while the photosynthesis-irradiance response curve of *C. praecox* decreased sharply.

The highest P_N values of *C. chinensis* and *C. praecox* were found under the experimental conditions of 27.3 and 25.3 °C, respectively (Fig. 1*C*). T_{leaf} of *C. chinensis* was approximately 1–2 °C higher than that of *C. praecox* from 08:00 to 15:00.

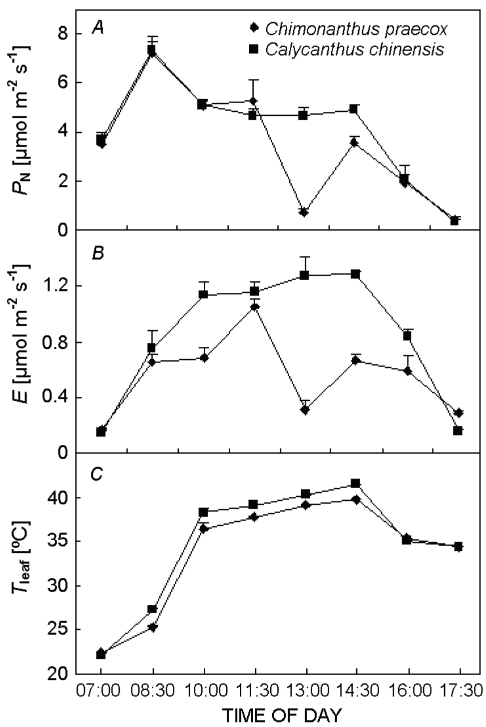

Fig. 1. Curves of diurnal variation of (*A*) net photosynthetic rate, P_N, (*B*) transpiration rate, E, and (*C*) leaf temperature, T_{leaf}.

Chl contents and fluorescence: There was a significant difference in the Chl content between both species (Fig. 3*A*). Both Chl *a* (p<0.05) and Chl *b* (p<0.05) contents in *C. chinensis* were nearly 33 % higher than those in *C. praecox*, while no difference was found in Chl *b*/*a* ratio (p>0.05).

F_v/F_m, F_0, and F_m at p<0.05 were all smaller in *C. praecox* than in *C. chinensis* (Fig. 3*B*). q_P, as an indication of the proportion of open photosystem 2 (PS2) reaction centres (RCs), did not differ between the two species (p>0.05). However, q_{NP} (p<0.05) in *C. praecox* was much higher than that in *C. chinensis*.

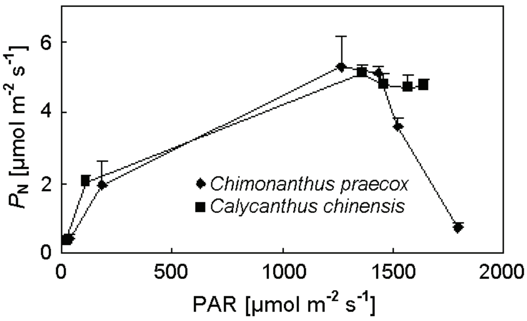

Fig. 2. The photosynthesis (P_N)-irradiance (PAR) response curves in leaves of *Chimonanthus* and *Calycanthus*.

Discussion

Photosynthesis: Both low temperature and high temperature affect photoinhibition to various degrees by restraining the enzymatic reactions of electron transport and carbon metabolism, as well as the stroma-grana lateral diffusion and protein synthesis involved in repair of damaged PS2 centres (Bertamini *et al*. 2006a,b). In our study, the phenomenon of photosynthetic midday depression was obviously different between the two species. The response of leaf photosynthesis to T_{leaf} and PAR suggested that the optimum environment for *C. chinensis* was under T_{leaf} of 27.34 °C with the PAR of 1 366.22 μmol m^{-2} s^{-1} while for *C. praecox* was under 25.29 °C with 1 265.63 μmol m^{-2} s^{-1}. Compared with *C. praecox*, *C. chinensis* had a lower CI, which met well with the results of Xu *et al.* (1997) and a higher SI. But the similar P_S (P_N at SI) of the two species meant that there was no difference in photosynthetic ability. Li *et al.* (2000) report that P_N of *C. praecox* is high and can accommodate a wide range of PAR with a good shade-endurance. However, our observation showed that *C. chinensis* had more advantages in obtaining and utilizing photon energy than *C. praecox*. This phenomenon was in agreement with another observation (Zhou and Ye 2002), which stated that *C. chinensis* grew well at the edge of forest or on open hills, while the species growing in the forest became the underbrush or tended to die, *e.g.* in Suwu, Zhuchuan of Lin'an City in China.

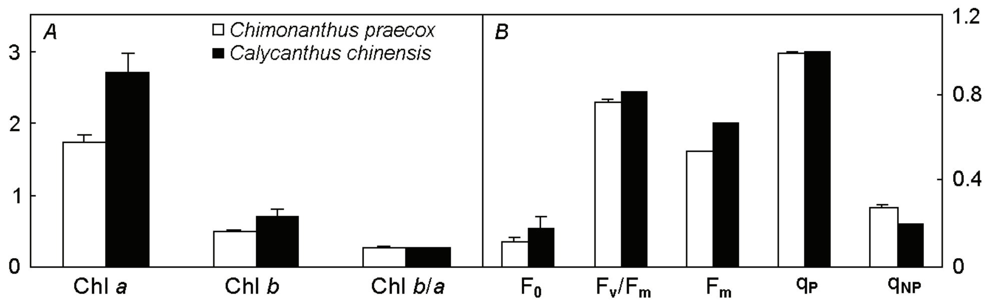

Fig. 3. The comparison of (*A*) chlorophyll (Chl) content and (*B*) Chl fluorescence parameters in *Chimonanthus* and *Calycanthus*.

Chl content is one of the most important factors in determining the rate of photosynthesis (Garty *et al.* 2001) and dry matter production (Ghosh *et al.* 2004). Chl content also influences fluorescence, because the relative number of antenna pigments that transfer excitation energy to the RC of PS2 and the efficiency of energy transfer between antenna and RC are critical factors in the balance between optimizing energy conversion and avoiding photodamage (Vasil'ev *et al.* 2004). According to our results, both contents of Chl *a* and *b* of *C. chinensis* were much greater than those of *C. praecox*, which showed that *C. chinensis* had more advantages in obtaining energy. But no significant difference of the two species in Chl *b*/*a* ratio suggested that the habitats of the two were similar (Bertamini *et al.* 2006a). The appreciably lower Chl *b*/*a* ratio in *C. chinensis* implied that it had a little bit more sun-endurance than *C. praecox* (Feng *et al.* 2004, Baig *et al.* 2005, Xu *et al.* 2005).

Chl fluorescence: The fluorescence emission depends on the pigment content, the excitation irradiance, and the fluorescence yield or efficiency of fluorescence emission (Campbell *et al.* 1998). F_0 is the emission of Chl before PS2 induction, and its magnitude relates to the Chl content (Lu *et al*. 1994, Mauromicale *et al.* 2006). Decrease in F_0 is associated with an increased capacity for energy dissipation within light-harvesting complexes (Demmig-Adams 1990, Yang *et al*. 2004), while increase in F_0 is related to partly reversible inactivation or irreversible damage in the RCs of PS2 (Yamane *et al*. 1997, Yang *et al*. 2004). According to Genty *et al*. (1989) and Pastenes and Hoton (1996), Φ_{PS2} is proportional to the product of q_P and the efficiency of excitation capture by open PS2 RCs, denoted as F_v/F_m. In this study, F_v/F_m values of the two species were both about 0.8, which means leaves measured were not threatened or hardly changed (Lichtenthaler 1988). F_m reflects the electron transport from Q_A (the redox state of the quinone acceptor A in PS2) to the electron transfer chain (Ortiz and Cardemil 2001). When leaves measured were not threatened, the two species showed no significant variations in F_0, and F_m in *C. chinensis* was observably higher than that in *C. praecox*, showing that electron transport was discriminative.

The q_P reflects the electron pressure at the Q_A (Holtgrefe *et al*. 2003) and the balance between excitation of PS2 RCs. High q_P is advantageous for the separation of electric charge in the RC, and the ability to transport electrons and the quantum yield of PS2 are enhanced (Guo *et al*. 2006). According to our results, the values of q_P of the two species were both about 1.0. Hence the two species may have no difference in the activities of electron transport in PS2.

Processes of q_{NP} protect against the irradiation-induced inhibition of photosynthesis, which can arise as a consequence of the excessively reduced state of the primary quinone acceptor of electrons from PS2 (Mohotti and Lawlor 2002). The resulting surplus of energy becomes a potential source of damage to the plant (Melis 1999), as it can lead to formation of destructive oxidative molecules, which can damage the photosynthetic apparatus in photoinhibition (Aro *et al*. 1993), and the q_{NP} can represent the energy dissipated as heat energy (Vasil'ev *et al*. 1998, Veres *et al*. 2006), which can not be utilized to transport photosynthetic electrons. The higher q_{NP} in *C. praecox* showed that the energy absorbed in the physiological range of irradiances might be much higher than photochemical utilization. The lower q_{NP} in *C. chinensis* indicated that this species can effectively reduce the thermal dissipation and utilize energy absorbed by antenna pigments in PS2 for photosynthesis (Guo *et al*. 2006). Thus, compared with *C. praecox*, *C. chinensis* was more appropriate to the thermal dissipation and had more advantages under the same irradiance.

In conclusion, compared with *C. praecox*, *C. chinensis* had a lower CI, a higher SI, and a higher Chl content, and was more adaptive to higher temperature and propitious to the thermal dissipation. In addition, *C. chinensis* produces abundant seed, has a higher germination rate, with a wider adaptability to temperature (Zhang *et al*. 2001). All these facts suggest that *C. chinensis* is viable and can be extended-cultivated as an ornamental plant in Eastern China. And it seems *C. chinensis* will no longer need to be on the endangered-plant list of the national second-grade protection in China.

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