Evaluate the photosynthesis and chlorophyll fluorescence of *Epimedium brevicornu* Maxim

Jian Zaiyou¹,², Tang Xiaomin², Wang Hongsheng¹ & Xu Guifang¹

The diurnal variation of photosynthesis, light response curve and CO₂ response curve in *Epimedium brevicornu* Maxim leaves were determined with Li-6400 photosynthesis system to evaluate the photosynthesis of *E. brevicornu*. Fluorescence of chlorophyll in the leaves were determined with PAM-2500 portable chlorophyll fluorescence apparatus in the study. The results showed that the midday depression of photosynthesis was very obvious in the *E. brevicornu* leaves. The light compensation point of *E. brevicornu* leaves was about 15 µmol m⁻² s⁻¹. The light saturation point of *E. brevicornu* leaves was below 800 µmol m⁻² s⁻¹, which was lower than the general sunlight intensity at noon in summer. The CO₂ saturation point of *E. brevicornu* leaves was much higher than the content of CO₂ in general air. *E. brevicornu* was a typical shade plant and could survive in very low sunlight. *E. brevicornu* could not endure strong sunlight and high air temperature. The net photosynthetic rate of *E. brevicornu* leaves linearly correlated with the content of CO₂ in the leaf chamber when the content was below CO₂ saturation point. *E. brevicornu* possessed great potential of photosynthesis.

As a kind of traditional Chinese medicine with aphrodisiac, anti-rheumatic and tonic effects, epimedii folium was usually used to cure impotence, emission, osteomalacia, rheumatism, apoplexy and so on¹. Epimedium folium was dried leaves preparation from *Epimedium brevicornu* Maxim, *E. pubescens* Maxim, *E. sagittatum* (Sich. et Zucc.) Maxim or *E. koreum* Nakai¹. There were some medicinal chemical components such as Icariin, Caohuoside, Baohuoside, Epimedin A, Epimedin B and Epimedin C in Epimedioli folium²,³.

Epimedioli folium came from wild resources in the past times. The wild Epimedioli folium resources were sharply decreasing because of the increased demand on them and the change of environment. The plants in *Epimedium* are herbaceous perennial⁴. The root and the rhizome of these plants can grow for several years although their leaves wither in winter. The roots and rhizomes of these plants were usually dug out by people because there are certain content of medicinal chemical components in them. Therefore, Epimedioli folium resources were seriously destroyed. To satisfy the needs of patient and protect wild Epimedioli folium resources, the plants of Epimedioli folium should be bred and cultivated.

The diurnal variation of photosynthesis, light response curve, CO₂ response curve and chlorophyll fluorescence characteristics of *E. brevicornu* leaves were determined in this study to define the suitable growing conditions and provide evidence in support of cultivating *E. brevicornu*. The results contribute to studying the cultivation of *E. brevicornu*, satisfying the needs of patients on Epimedioli folium and protecting the wild *E. brevicornu* resources.

Results

The results of the diurnal variation of photosynthesis in *E. brevicornu* leaves are shown in Table 1. The diurnal variation curve of photosynthesis in the leaves is drawn in Fig. 1.

There is obvious midday depression in the diurnal variation of photosynthesis of *E. brevicornu* leaves. They commonly photosynthesized in the morning and evening on sunny days in summer. The photosynthesis nearly stopped at noon when the air temperature was high and the sunlight was intense. *E. brevicornu* leaves did not endure the strong sunlight and high temperature.

The results of light response curves of photosynthesis in the leaves are shown in Tables 2 and 3. The fitted light response curve of photosynthesis in the leaves with the average fitted indexes in each repeat of light response curve determination is shown in Fig. 2.

*E. brevicornu* leaves were able to survive in very low sunlight such as 15 µmol m⁻² s⁻¹. The photosynthesis in the leaves quickly increased along with the increase of light intensity when the light intensity was above the light
compensation point. The light saturation point was much lower than the intensity of direct sunlight in summer. Strong sunlight inhibited the photosynthesis in *E. brevicornu* leaves. The results of CO₂ response curve of photosynthesis in the leaves are shown in Tables 3 and 4. The fitted CO₂ response curve of photosynthesis with the average fitted indexes in each repeat of CO₂ response curve determination is shown in Fig. 3. The CO₂ compensation point was about 48 μmol mol⁻¹, which was much lower than the content of CO₂ in air in field. The photosynthesis in *E. brevicornu* leaves was approximately linear to the content of CO₂ in the leaf chamber. The CO₂ saturation point of the leaves was about 1766 μmol mol⁻¹, which was much higher than the content of CO₂ in air in field. *E. brevicornu* leaves possessed large potential of utilizing CO₂ in photosynthesis. The CO₂ with excessive content inhibited the photosynthesis in *E. brevicornu* leaves. The results of slow kinetics of chlorophyll fluorescence in the leaves are shown in Table 5.

| Repeat | Time | Photo | PARi | Tleaf | CO₂S | Trmmol | Cond | Ci |
|--------|------|-------|------|-------|------|--------|------|----|
| 1      | 7:12 | 0.0780| 108.5| 28.68 | 433.0| 0.0433 | 0.000578| 372.1|
|        | 8:01 | 0.0931| 176.8| 31.67 | 433.0| 0.0435 | 0.000153| 371.0|
|        | 9:01 | 0.1888| 628.7| 33.24 | 433.1| 0.0433 | 0.000578| 201.7|
|        | 10:13| 0.1070| 1460.9| 33.53 | 434.1| 0.1344 | 0.001138| 221.6|
|        | 11:04| 0.0615| 1578.3| 35.16 | 427.0| 0.1593 | 0.000865| 218.2|
|        | 12:00| 0.0377| 1667.6| 36.44 | 425.4| 0.2048 | 0.001061| 262.4|
|        | 13:15| 0.0853| 1598.9| 37.25 | 421.2| 0.2590 | 0.001659| 323.3|
|        | 14:04| 0.0276| 1554.4| 37.27 | 416.9| 0.1860 | 0.001404| 308.5|
|        | 15:00| 0.0140| 1446.8| 36.39 | 415.3| 0.0977 | 0.000652| 295.7|
|        | 16:00| 0.0752| 1163.1| 35.08 | 413.2| 0.0487 | 0.000439| 94.0 |
|        | 17:00| 0.1485| 934.4 | 34.01 | 411.8| 0.0586 | 0.000708| 45.7 |
|        | 18:00| 0.1017| 544.7 | 33.16 | 412.4| 0.0353 | 0.000545| 88.2 |
| 2      | 7:12 | 0.0813| 100.6 | 28.64 | 433.1| 0.0415 | 0.000558| 386.0|
|        | 8:01 | 0.1014| 180.7 | 31.65 | 433.0| 0.0469 | 0.000596| 171.4|
|        | 9:01 | 0.2082| 589.5 | 33.26 | 433.0| 0.1358 | 0.000909| 171.4|
|        | 10:13| 0.1086| 1432.3| 33.47 | 435.1| 0.0965 | 0.000773| 304.4|
|        | 11:04| 0.0642| 1578.4| 35.17 | 427.0| 0.1590 | 0.000864| 213.7|
|        | 12:00| 0.0400| 1668.4| 36.45 | 425.5| 0.2057 | 0.001063| 259.1|
|        | 13:15| 0.0089| 1555.1| 37.23 | 421.0| 0.2527 | 0.002035| 338.5|
|        | 14:04| 0.0376| 1554.3| 37.27 | 416.9| 0.1857 | 0.001434| 300.6|
|        | 15:00| 0.0185| 1349.0| 36.51 | 414.5| 0.0965 | 0.000773| 304.4|
|        | 16:00| 0.0779| 1153.2| 35.08 | 413.1| 0.0512 | 0.000456| 94.5 |
|        | 17:00| 0.1552| 933.5 | 34.01 | 411.8| 0.0599 | 0.000735| 44.2 |
|        | 18:00| 0.1037| 543.9 | 33.15 | 412.4| 0.0356 | 0.000549| 84.3 |
| 3      | 7:12 | 0.0855| 105.5 | 28.56 | 433.3| 0.0437 | 0.000556| 220.4|
|        | 8:01 | 0.1086| 178.8 | 31.65 | 433.0| 0.0363 | 0.000532| 386.1|
|        | 9:01 | 0.2234| 591.9 | 33.26 | 433.0| 0.0437 | 0.000556| 220.4|
|        | 10:13| 0.1122| 1460.8| 33.53 | 434.0| 0.1333 | 0.001142| 216.0|
|        | 11:04| 0.0689| 1634.3| 35.59 | 425.6| 0.1294 | 0.000783| 201.7|
|        | 12:00| 0.0488| 1667.9| 36.44 | 425.4| 0.2057 | 0.001064| 247.9|
|        | 13:15| 0.0093| 1598.9| 37.25 | 421.2| 0.2595 | 0.001651| 319.0|
|        | 14:04| 0.0432| 1530.9| 37.07 | 417.4| 0.2176 | 0.001347| 276.4|
|        | 15:00| 0.0228| 1397.9| 36.32 | 414.8| 0.0999 | 0.000889| 308.7|
|        | 16:00| 0.0859| 1147.0| 35.09 | 413.2| 0.0517 | 0.000458| 69.4 |
|        | 17:00| 0.1679| 934.5 | 34.01 | 411.8| 0.0584 | 0.000711| 6.2  |
|        | 18:00| 0.1051| 571.0 | 33.71 | 412.5| 0.0390 | 0.000498| 46.1 |
|        | 19:00| 0.0706| 58.4 | 32.26 | 415.0| 0.0368 | 0.000738| 236.0|

Table 1. Diurnal variations in *Epimedium brevicornu* leaves photosynthesis. Photo, photosynthetic rate, unit: μmol CO₂ m⁻² s⁻¹. PARi, in-chamber quantum sensor, unit: μmol m⁻² s⁻¹. Tleaf, temperature of leaf thermocouple, unit: °C. CO₂S, sample cell CO₂, unit: μmol mol⁻¹. Trmmol, transpiration rate, unit: mmol H₂O m⁻² s⁻¹. Cond, conductance to H₂O, unit: mol H₂O m⁻² s⁻¹. Ci, intercellular CO₂ concentration, unit: μmol CO₂ mol⁻¹.
The slow kinetics of chlorophyll fluorescence of *E. brevicornu* leaves indicates that the maximal photochemical efficiency of photosystem II (\((F_m - F_o)/F_m\)) and the ETR of photosystem II in them were all very low. The fraction of energy dissipated as heat via the regulated photoprotective NPQ mechanism (\(Y(NPQ)\)) was much more than that passively dissipated in the form of heat and fluorescence (\(Y(NO)\)).

The results of rapid light curves of chlorophyll fluorescence in *E. brevicornu* leaves are shown in Tables 5 and 6. The fitted rapid light curve of chlorophyll fluorescence with the average fitted indexes in each repeat of rapid light curve is shown in Fig. 4.

The fitted maximum electron transport rate in the leaves reached 22.2 µmol m\(^{-2}\) s\(^{-1}\), which was much higher than ETR of photosystem II in slow kinetics of chlorophyll fluorescence. The leaves possessed large potential of photosynthesis.

**Discussion and conclusion**

There was obvious midday depression in the photosynthesis of *E. brevicornu* leaves. The photosynthesis nearly stopped at noon when the direct sunlight was intense. Therefore, *E. brevicornu* could not endure strong sunlight and high air temperature. There was obvious midday depression in the photosynthesis of *E. pseudowushanense* also\(^6\). The midday depression in the photosynthesis of *E. brevicornu* leaves was related to sunlight and air temperature\(^5\). The net photosynthetic rate of *E. brevicornu* leaves significantly reduced when the sunlight intensity was above 1000 µmol m\(^{-2}\) s\(^{-1}\). There was little variation in sunlight intensity from 11:00 to 14:00 in summer. The net photosynthetic rates of *E. brevicornu* leaves was the lowest at 13:00 in summer because the air temperature was the highest at this time. The light compensation point of the leaves was about 15 µmol m\(^{-2}\) s\(^{-1}\), which indicated that *E. brevicornu* could survive in very low sunlight. The study of Liu et al. showed that the light compensation point of *E. sagittatum* (Sieb. & Zucc.) Maxim leaves was 13–17 µmol m\(^{-2}\) s\(^{-1}\), which was consistent with the result in this study. The result of WANG et al. that the light compensation point of *E. sagittatum* was about 3.6 µmol m\(^{-2}\) s\(^{-1}\) seemed not reasonable\(^5\). The light saturation point in the light response curve of *E. brevicornu* leaves was lower than general sunlight intensity at noon in summer. Wild *E. brevicornu* grew in forests or shady slope and was rarely seen in the open places in general. Therefore, *E. brevicornu* is a typical shade plant. Luo et al. studied the characteristics of photosynthesis in *E. koreanum* Nakai and found that the photosynthetic rate was the highest in 70% light transmittance\(^10\). *E. brevicornu* should be properly shaded when cultivated.

It was indicated in CO\(_2\) response curve in *E. brevicornu* leaves that the CO\(_2\) saturation point was about 1766 µmol mol\(^{-1}\), which was much higher than the content of CO\(_2\) in general air. Therefore, *E. brevicornu* is characteristic of C\(_3\) plant. The net photosynthetic rate of *E. brevicornu* leaves was linearly correlated with the content of CO\(_2\) in air when the leaf chamber when it was below CO\(_2\) saturation point. This is consistent with the study of Wang Xujun on *E. sagittatum*\(^9\). The results indicated that there was very great potential to utilize CO\(_2\) in *E. brevicornu*.

The characteristics of chlorophyll fluorescence in *E. brevicornu* showed that it possessed great potential of photosynthesis. It is prospective to breed a new breed with high photosynthetic rate and yield.

**Materials and methods**

All methods were performed in accordance with the local relevant guidelines, regulations and legislation.

**Instruments.** LI-6400 photosynthesis system (LI-6400 Inc., Lincoln, NE, USA) and PAM-2500 portable chlorophyll fluorescence apparatus (PAM-2500, Walz, Germany) were used in the study.

**Materials.** About 90 living *E. brevicornu* plants were collected from Taihang Mountains in October 2018. The *E. brevicornu* was not in endangered or protected. The collection of these *E. brevicornu* plants was permitted by local government. These plants were averagely planted in nine plots of 2 m\(^2\). The roots of *E. pubescens* were planted 6–8 cm below ground. These plots were placed on farmland near Taihang Mountains and covered with sunshade net (about 70% light transmittance). These plants were timely irrigated after planting to ensure that they grew well but not fertilized.

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**Figure 1.** Diurnal variations in *Epimedium brevicornu* leaves photosynthesis.
Determination of photosynthetic characteristics. The photosynthetic characteristics of mature leaves on the *Epimedium brevicornu* plants were determined between June 6–8, 2019 with the Li-6400 photosynthesis system. The diurnal variation of photosynthesis in three leaves of three plants was determined. When the light response curve was determined, the temperature of the leaf chamber was set at 28 °C, and the concentration of CO₂ in the leaf chamber was set at 400 µmol mol⁻¹. When determining the CO₂ response curve, the light intensity in the leaf chamber was set at 1000 µmol m⁻² s⁻¹, and the temperature of the leaf chamber was set at 28 °C. The light response curve and CO₂ response curve were determined three times in three leaves of three different plants.

Determination of chlorophyll fluorescence characteristics. The fluorescence characteristics of chlorophyll in *Epimedium brevicornu* leaves were determined with PAM-2500 portable chlorophyll fluorescence apparatus between June 8–9, 2019. The leaves underwent dark adaptation for 30 min before determining slow kinetics of

| Repeat | PARi | Photo | Fitted | CO₂S | Transmol | Tleaf | Cond | Ci |
|--------|------|-------|--------|------|----------|-------|------|----|
| 1      |      |       |        |      |          |       |      |    |
| 2000.5 | 2.005| 1.897 | 400.3  | 0.670| 28.07    | 0.0496| 326.6|
| 1800.1 | 2.108| 2.050 | 399.6  | 0.623| 28.09    | 0.0450| 315.6|
| 1498.9 | 2.147| 2.272 | 400.2  | 0.640| 27.93    | 0.0311| 277.4|
| 1399.9 | 2.177| 2.342 | 400.1  | 0.539| 28.10    | 0.0380| 299.3|
| 1001.2 | 2.684| 2.600 | 399.5  | 0.491| 28.12    | 0.0340| 264.1|
| 801.5  | 2.705| 2.702 | 400.2  | 0.454| 27.86    | 0.0369| 245.4|
| 600.8  | 2.736| 2.765 | 399.8  | 0.442| 28.14    | 0.0381| 245.2|
| 399.6  | 2.699| 2.738 | 400.3  | 0.416| 28.14    | 0.0280| 242.6|
| 199.7  | 2.570| 2.419 | 399.8  | 0.396| 28.14    | 0.0264| 235.2|
| 150.8  | 2.250| 2.205 | 399.3  | 0.405| 28.14    | 0.0268| 238.1|
| 100.6  | 1.828| 1.833 | 400.1  | 0.387| 28.15    | 0.0254| 276.1|
| 49.6   | 0.925| 1.092 | 399.9  | 0.400| 28.15    | 0.0261| 333.7|
| 20.5   | 0.341| 0.266 | 400.7  | 0.373| 28.15    | 0.0242| 368.4|
| 2000.1 | 2.171| 2.077 | 400.1  | 0.809| 28.09    | 0.0543| 326.2|
| 1800.1 | 2.374| 2.350 | 400.0  | 0.719| 28.11    | 0.0477| 297.1|
| 1499.9 | 2.720| 2.713 | 399.6  | 0.678| 28.12    | 0.0448| 274.9|
| 1199.1 | 2.777| 2.991 | 400.0  | 0.626| 28.13    | 0.0411| 284.3|
| 999.3  | 3.008| 3.102 | 399.7  | 0.560| 28.14    | 0.0364| 257.9|
| 799.8  | 3.213| 3.120 | 400.5  | 0.531| 28.15    | 0.0345| 280.7|
| 601.0  | 3.073| 2.995 | 400.4  | 0.486| 28.16    | 0.0314| 255.4|
| 399.7  | 2.688| 2.617 | 399.5  | 0.462| 28.16    | 0.0299| 267.7|
| 199.7  | 1.904| 1.753 | 399.8  | 0.414| 28.15    | 0.0269| 277.0|
| 149.8  | 1.241| 1.404 | 400.8  | 0.230| 27.97    | 0.0072| 330.0|
| 100.7  | 0.837| 0.978 | 400.3  | 0.246| 28.05    | 0.0117| 334.3|
| 49.8   | 0.338| 0.425 | 399.9  | 0.256| 27.97    | 0.0123| 285.6|
| 20.5   | 0.156| 0.040 | 399.4  | 0.281| 28.06    | 0.0133| 335.2|
| 1999.9 | 0.952| 0.898 | 484.7  | 0.944| 28.79    | 0.0113| 297.0|
| 1800.6 | 1.226| 1.201 | 483.3  | 0.964| 28.20    | 0.0135| 293.4|
| 1599.8 | 1.423| 1.497 | 480.8  | 0.955| 28.35    | 0.0158| 298.1|
| 1400.8 | 1.751| 1.779 | 477.7  | 0.925| 27.84    | 0.0179| 288.3|
| 1200.6 | 2.004| 2.046 | 474.3  | 0.892| 28.51    | 0.0202| 287.0|
| 1001.2 | 2.230| 2.286 | 471.3  | 0.822| 28.29    | 0.0217| 282.7|
| 800.5  | 2.570| 2.485 | 468.6  | 0.751| 28.13    | 0.0233| 285.1|
| 600.7  | 2.676| 2.607 | 465.8  | 0.678| 28.01    | 0.0249| 288.0|
| 399.7  | 2.493| 2.571 | 463.7  | 0.713| 28.24    | 0.0301| 305.6|
| 200.2  | 2.164| 2.109 | 463.1  | 0.779| 27.87    | 0.0330| 341.7|
| 149.6  | 1.823| 1.825 | 462.3  | 0.793| 27.55    | 0.0317| 353.1|
| 99.5   | 1.420| 1.398 | 461.4  | 0.751| 28.16    | 0.0299| 367.9|
| 49.7   | 0.617| 0.725 | 461.2  | 0.702| 28.26    | 0.0286| 409.2|
| 20.8   | 0.195| 0.136 | 458.8  | 0.523| 28.11    | 0.0194| 423.4|

Table 2. Light response curves of *Epimedium brevicornu* leaves photosynthesis. Photo, photosynthetic rate, unit: µmol CO₂ m⁻² s⁻¹. PARi, in-chamber quantum sensor, unit: µmol m⁻² s⁻¹. Tleaf, temperature of leaf thermocouple, unit: °C. CO₂S, sample cell CO₂, unit: µmol mol⁻¹. Transmol, transpiration rate, unit: mmol H₂O m⁻² s⁻¹. Cond, conductance to H₂O, unit: mol H₂O m⁻² s⁻¹. Ci, intercellular CO₂ concentration, unit: µmol CO₂ mol⁻¹.
chlorophyll fluorescence. Then the light curves of chlorophyll fluorescence were determined. All of these determinations were repeated three times on three mature leaves of three plants.

The data was analysed with SPSS (Statistical Product and Service Solutions, International Business Machines Corporation, USA). The light response curves were fitted with following modified rectangular hyperbola model11,12.

\[
\text{Photo} = E \cdot (1 - M \cdot \text{PAR}) \cdot (\text{PAR} - \text{LCP})/(1 + N \cdot \text{PAR})
\]

PAR is the value of light intensity in leaf chamber of Li-6400 photosynthesis system. Photo is net photosynthetic rate. LCP is the light compensation point. E is the apparent quantum yield. M and N are parameters. The dark respiration rate under the LCP is calculated according to E\cdot LCP. The light saturation point (LSP) is calculated according to (((M + N) \cdot (1 + N\cdot \text{LCP})/M)\frac{1}{2}) /−1)/N.

The net photosynthetic rate under the light saturation point (LSP) can be calculated according to the above model.

The CO₂ response curves were fitted with below modified rectangular hyperbola model11,12.

\[
\text{Photo} = E \cdot (1 - M \cdot \text{PAR}) \cdot (\text{PAR} - \text{CCP})/(1 + N \cdot \text{PAR})
\]

PAR is the value of light intensity in leaf chamber of Li-6400 photosynthesis system. Photo is net photosynthetic rate. CCP is CO₂ compensation point. E is also the apparent quantum yield. M and N are parameters. The dark respiration rate under the CO₂ calculated according to E\cdot CCP. The CO₂ saturation point (CSP) is calculated according to (((M + N) \cdot (1 + N\cdot \text{CCP})/M)\frac{1}{2}) /−1)/N.

| Project | Repeat | E       | M       | N       | LCP (CCP) | LSP (CSP) | E\cdot LCP (E\cdot CCP) | PLSP (PCSP) | R²   |
|---------|--------|---------|---------|---------|-----------|-----------|-------------------------|-------------|------|
| Light response | 1   | 0.0536  | 0.000230 | 0.01465 | 14.017    | 534.52    | 0.751                   | 2.771       | 0.979|
|         | 2   | 0.01496 | 0.000304 | 0.002298| 17.67     | 863.54    | 0.264                   | 3.127       | 0.987|
|         | 3   | 0.0284  | 0.000389 | 0.00647 | 15.314    | 525.84    | 0.435                   | 2.620       | 0.993|
|         | Average | 0.03232 | 0.000308 | 0.007806| 15.667    | 641.30    | 0.483                   | 2.839       | 0.986|
| CO₂ response | 1   | 0.01113 | 0.000309 | 0.000001| 35.56     | 1634.6    | 0.396                   | 8.794       | 0.973|
|         | 2   | 0.01011 | 0.0002707| 0.000001| 51.16     | 1871.0    | 0.517                   | 9.063       | 0.982|
|         | 3   | 0.01049 | 0.000283 | 0.000001| 58.72     | 1794.6    | 0.616                   | 8.945       | 0.988|
|         | Average | 0.010577| 0.000288 | 0.000001| 48.48     | 1766.7    | 0.5097                  | 8.934       | 0.981|

Table 3. Results of fitting light response curves and CO₂ response curves. LCP is light compensation point, unit: µmol m⁻² s⁻¹. CCP CO₂ compensation point, unit: µmol mol⁻¹. CSP is CO₂ saturation point, unit: µmol mol⁻¹. LSP is light saturation point, unit: µmol m⁻² s⁻¹. PLSP is the net photosynthetic rate at the light saturation point, unit: µmol m⁻² s⁻¹.
Table 4. CO₂ response curves of *Epimedium pubescens* leaves photosynthesis. Photo, photosynthetic rate, unit: µmol CO₂ m⁻² s⁻¹. PARᵢ, in-chamber quantum sensor, unit: µmol m⁻² s⁻¹. Tleaf, temperature of leaf thermocouple, unit: °C. CO₂S, sample cell CO₂, unit: µmol mol⁻¹. Trmmol, transpiration rate, unit: mmol H₂O m⁻² s⁻¹. Cond, conductance to H₂O, unit: mol H₂O m⁻² s⁻¹. Ci, intercellular CO₂ concentration, unit: μmol CO₂ mol⁻¹.

| Repeat | CO₂S | Photo | Fitted | Trmmol | Tleaf | PARᵢ | Cond | Ci   |
|--------|------|-------|--------|--------|-------|-------|------|------|
| 2563.5 | 5.3565 | 5.834 | 1.043   | 28.09  | 1000.9  | 0.0488 | 2104.6     |
| 1899.2 | 9.3139 | 8.553 | 0.894   | 28.13  | 1001.3  | 0.0411 | 1473.1     |
| 1695.6 | 9.6047 | 8.781 | 0.923   | 28.14  | 1001.3  | 0.0426 | 1277.7     |
| 1398.7 | 7.8518 | 8.603 | 0.971   | 28.24  | 1001.2  | 0.0445 | 1068.9     |
| 1099.0 | 7.5999 | 7.808 | 1.012   | 28.07  | 1001.0  | 0.0474 | 825.9      |
| 999.8  | 7.2034 | 7.409 | 1.012   | 28.18  | 1000.8  | 0.0468 | 720.1      |
| 800.2  | 6.1402 | 6.401 | 1.038   | 28.24  | 1000.8  | 0.0478 | 567.8      |
| 600.3  | 5.3804 | 5.117 | 1.020   | 28.06  | 1001.0  | 0.0479 | 400.5      |
| 400.5  | 3.4728 | 3.558 | 0.966   | 28.17  | 1000.6  | 0.0447 | 262.8      |
| 311.7  | 2.3457 | 2.777 | 0.908   | 28.13  | 1000.5  | 0.0420 | 212.2      |
| 139.4  | 1.5264 | 1.106 | 0.325   | 29.06  | 1199.8  | 0.0112 | 80.5       |
| 91.2   | 0.7625 | 0.602 | 0.856   | 29.55  | 1200.6  | 0.0185 | 20.9       |

| Repeat | CO₂S | Photo | Fitted | Trmmol | Tleaf | PARᵢ | Cond | Ci   |
|--------|------|-------|--------|--------|-------|-------|------|------|
| 2567.3 | 7.3494 | 7.740 | 0.741   | 28.10  | 1000.4  | 0.0345 | 1987.4     |
| 1897.3 | 9.4502 | 9.061 | 0.667   | 28.11  | 1000.1  | 0.0309 | 1295.6     |
| 1696.8 | 9.7506 | 9.880 | 0.682   | 28.11  | 1000.3  | 0.0316 | 1149.0     |
| 1394.4 | 8.3402 | 8.453 | 0.690   | 28.09  | 1000.2  | 0.0321 | 937.5      |
| 1099.3 | 7.4384 | 7.435 | 0.733   | 28.10  | 1000.3  | 0.0341 | 715.3      |
| 1000.5 | 6.8615 | 6.991 | 0.712   | 28.09  | 1000.2  | 0.0332 | 637.9      |
| 800.4  | 5.6185 | 5.929 | 0.720   | 28.09  | 1000.3  | 0.0336 | 507.4      |
| 601.4  | 4.2436 | 4.654 | 0.697   | 28.09  | 1000.2  | 0.0325 | 373.7      |
| 399.8  | 2.7830 | 3.142 | 0.641   | 28.08  | 1000.1  | 0.0298 | 238.1      |
| 310.1  | 2.2032 | 2.397 | 1.014   | 28.22  | 1000.6  | 0.0468 | 224.3      |
| 113.4  | 1.4361 | 0.610 | 0.322   | 28.00  | 1200.3  | 0.0111 | 95.2       |
| 88.8   | 0.3868 | 0.371 | 0.920   | 27.89  | 1200.9  | 0.0561 | 75.4       |

| Repeat | CO₂S | Photo | Fitted | Trmmol | Tleaf | PARᵢ | Cond | Ci   |
|--------|------|-------|--------|--------|-------|-------|------|------|
| 2564.3 | 6.0075 | 7.192 | 0.696   | 28.08  | 1000.3  | 0.0342 | 2016.5     |
| 1898.3 | 8.6724 | 8.913 | 0.609   | 28.09  | 1000.7  | 0.0477 | 1352.5     |
| 1696.5 | 9.6606 | 8.917 | 0.585   | 28.09  | 1000.1  | 0.0325 | 1077.5     |
| 1399.2 | 8.1282 | 8.482 | 0.698   | 28.10  | 1000.3  | 0.0324 | 952.8      |
| 1099.7 | 7.3780 | 7.513 | 0.705   | 28.14  | 999.9   | 0.0303 | 805.4      |
| 999.7  | 6.6075 | 7.071 | 0.676   | 28.04  | 1000.2  | 0.0388 | 685.7      |
| 800.3  | 6.0660 | 6.012 | 1.040   | 28.17  | 1001.1  | 0.0483 | 572.3      |
| 600.4  | 4.7405 | 4.714 | 1.028   | 28.18  | 1000.7  | 0.0476 | 421.0      |
| 413.5  | 3.2650 | 3.285 | 1.003   | 28.14  | 1000.9  | 0.0466 | 287.5      |
| 311.0  | 2.1327 | 2.413 | 0.974   | 28.14  | 1001.0  | 0.0452 | 225.0      |
| 202.1  | 1.0168 | 1.418 | 0.866   | 28.13  | 1199.8  | 0.0340 | 146.6      |
| 105.2  | 0.6105 | 0.473 | 0.750   | 28.03  | 1200.5  | 0.0199 | 51.2       |
| 83.9   | 0.0484 | 0.323 | 0.992   | 28.75  | 1200.8  | 0.0324 | 77.5       |

Figure 3. Fitted CO₂ response curve of *Epimedium pubescens* leaf photosynthesis with average indexes.
The net photosynthetic rate under the CO₂ saturation point (CSP) can be alternatively calculated according to the above model.

The light curves of chlorophyll fluorescence were fitted according to the below model of Eilers and Peeters\textsuperscript{12,13}.

\[ \text{ETR} = \frac{\text{PAR}}{a \cdot \text{PAR}^2 + b \cdot \text{PAR} + c} \]

ETR is the electron transport rate of photosynthetic system II. PAR is fluorescence intensity. The letters a, b and c are parameters.
Data availability
Data have been permanently archived: https://zenodo.org/record/4106097#.X41VfNSF7Gg.

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Table 6. Results of fitting rapid light curves of chlorophyll fluorescence in Epimedium pubescens leaves. \( \frac{f_{v}/f_{m}}{ETR \text{ factor}/2} \) is the maximum quantum yield of photosynthetic system II with a saturated pulse after dark adaptation. Alpha is the initial slope. ETRmax is the maximum electron transport rate. Ik is the minimum saturation of the light intensity.

| Index | Repeat 1 | Repeat 2 | Repeat 3 | Average |
|-------|----------|----------|----------|---------|
| \( \frac{f_{v}/f_{m}}{ETR \text{ factor}/2} \) | 0.241 | 0.236 | 0.226 | 0.2343 |
| Alpha | 0.043 | 0.046 | 0.06 | 0.0497 |
| ETRmax | 21.4 | 19.4 | 25.8 | 22.2 |
| Ik | 493.1 | 425.6 | 427.2 | 448.63 |
| \( \frac{(F_{m} - F_{o})}{F_{m}} \) | 0.5727 | 0.5616 | 0.5383 | 0.5575 |
| A | −0.000006637 | −0.000009711 | −0.000003762 | −0.00000379 |
| B | 0.06827 | 0.04571 | 0.05887 | 0.0576 |
| C | 11.5297 | 21.197 | 10.0040 | 14.24 |
| R² | 0.993 | 0.987 | 0.952 | 0.977 |

Figure 4. Fitted rapid light curve of chlorophyll fluorescence in Epimedium pubescens leaf with average indexes.
Author contributions
J.Z. Designed the study, implemented the experiment and wrote the manuscript. T.X. Participated in the experiment and data analysis. W.H. Participate in the experiment. X.G. Participate in the data analysis.

Competing interests
The authors declare no competing interests.

Additional information
Correspondence and requests for materials should be addressed to J.Z.

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