Photosynthetic characteristics and chlorophyll content of *Cypripedium japonicum* in its natural habitat

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**ABSTRACT**

This study characterizes the growth conditions of *Cypripedium japonicum* Thunb. (Korean lady’s slipper), a rare and endangered plant, across three different sites in its natural habitat. The three natural habitats of *C. japonicum* had different canopy densities that influenced the relative light intensity and quality (R/FR ratio) on the forest floor, the values of which, decreased with the increase in canopy density. The leaf mass per area of *C. japonicum* increased with an increase in canopy openness, and the difference in growth due to increased light availability was further confirmed by the chlorophyll content. Higher values of the average daily photosynthetic activity, transpiration rate, and stomatal aperture were recorded in *C. japonicum* growing in natural habitats that received a higher frequency of sunflecks. The activities of the photosystem and carbon fixation of the plants growing in the three habitats were compared through the light-response and A–Ci curves, and it was found that their photosynthetic capacity decreased in a low light environment. The growth and physiological characteristics of *C. japonicum* growing in different habitats were dependent on the light conditions in the stand, and therefore, increasing the light availability by control of canopy density may improve the propagation of *C. japonicum*. We believe that the findings of our study will facilitate the prediction of population dynamics and the long-term conservation and restoration of *C. japonicum*.

**Introduction**

*Cypripedium japonicum* Thunb. (Korean lady’s slipper), belongs to the *Cypripedium* genus of the Orchidaceae family, and is endemic to Northeast Asia, growing only in Korea, Japan, and China. *C. japonicum* is a rare plant in Korea that grows naturally in Gyonggi-do, including Gwangneung Forest, Jeollanam-do, and Jeollabuk-do. However, the population is small because individuals are scattered and damaged due to the over-collection for their unique and beautifully shaped leaves. *C. japonicum* is listed as critically endangered (CR) at the regional level (Korea), and endangered (EN) at the global level according to IUCN Red List criteria (KNA 2008; Pi et al. 2015). In particular, many species of Orchidaceae are on the verge of extinction due to rapid population declines because of habitat destruction, due to changes in land use, and overexploitation of their use as ornamental and medicinal plants (Swarts and Dixon 2009). In particular, high levels of harvesting and vegetation succession, which has been ongoing for many years, reduce orchid populations and affect natural habitats in Northeast Asian countries including China, Japan, and Korea (KNA 2017). *Cypripedium japonicum* is also on the verge of extinction, therefore extensive research and increased efforts are needed for its conservation (Lee et al. 2021). With a decrease in global biodiversity, decrease in the number, reintroduction, and preservation of endangered species are greatly needed (Swarts and Dixon 2009). Conservation of natural habitats and populations begins with the understanding of the environment, structure, and species composition in natural habitats, along with the relationships between various biotic and abiotic factors affecting natural populations (Shin et al. 2014). In the forest ecosystem, plants respond physiologically to environmental conditions, such as soil characteristics, temperature, light, and moisture. They live in areas suitable for their growth. The propagation vigor of orchids responds to internal factors, such as the size and age of the plant, and external environmental factors, such as light and temperature (de Jong et al. 1998). Because terrestrial orchids have underground structures that store resources produced during the previous year (Willems et al. 2001), the current vital rate of an orchid correlates with that of the previous year (Horvitz et al. 2010). Endangered orchids such as *C. japonicum* are greatly benefited by habitat management (Willems et al. 2001). Habitat management is mostly carried out by...
increasing the amount of available light by removing competing vegetation and creating forest gaps (Coates et al. 2006; Janečková et al. 2006). Higher light availability in natural habitats can promote an increase in the size of plants abundant flowering, and fruiting (Cho et al. 2019). Sunlight has a decisive influence on the metabolic activity and growth of plants in general. In the light conditions of the forest floor, the chlorophyll content of species with low shade tolerance decreases, and their growth is poor due to decrease in light absorption and photosynthesis (Kim et al. 2008). Since photosynthetic capacity is a good indicator for comparing plant stress under abiotic factors (Brestic and Zivcak 2013), it can provide useful information such as the estimation of plant growth rate and viability for the conservation of endangered species. Various studies have been conducted on C. japonicum in conservation biology including the investigation into the germination and mycorrhizal complex, which are widely investigated in Orchidaceae (Sim et al. 2010; Bae 2012; Lee et al. 2015), pollination vectors (Sun et al. 2009; Nam et al. 2014), natural habitat environments and floras (Park and Kim 1995; Seo et al. 2011), genetic characteristics and diversity (Chung et al. 2009; Kim et al. 2015; Tian et al. 2018), population dynamics (Cho et al. 2019; Lee et al. 2021), and population dynamics of C. japonicum specifically in relation to canopy density (Takayuki 2014; Pi et al. 2015). However, conclusive results of physiological studies on C. japonicum investigating into the effect of light conditions on metabolic activities have not yet been presented.

It is a known fact that the light availability on forest floor varies according to the canopy structure, and this can influence a change in the chlorophyll content and photosynthetic characteristics thereby affecting the photomorphogenesis of C. japonicum plants growing there. This study quantitatively evaluates the light conditions of natural habitats of C. japonicum in South Korea specifically in areas with different canopy structures and attempts to determine the corresponding photosynthetic capacity of the C. japonicum plants. Further, this investigation discusses the improvement of conservation and management practices for the propagation of C. japonicum.

Materials and methods

Experimental materials and natural habitat environment

The experimental materials of this study were healthy C. japonicum plants measured from their natural habitats in Pocheon-si (PC), Gyonggi-do; Chuncheon-si (CC), Gangwon-do; and Gwangyang-si (GY), Jeollanam-do (Figure 1). To investigate the canopy structure, light conditions, and consequential physiological responses in these sites, surveys were conducted at three different times of the year: in April, June, and August 2018.

The average temperature and relative humidity at each site were obtained over three days before and after photosynthesis measurements using temperature and humidity sensors (STL_T/RH, STA Corp., Anyang, Korea).

In each area, we selected a site that was expected to have a different canopy structure than other sites. The canopy structures of natural habitats were compared using canopy openness and breast height area (BHA). Canopy openness is the percentage of the total canopy area not covered by the leaf layer. To measure canopy openness, a camera (Nikon D300 camera with Sigma 4.5 mm lens; F2.8 EX DC CIRCULAR FISHEYE, Tokyo, Japan) was installed where C. japonicum plants were located in the natural habitats at a height of 1.5 m from the ground, and pictures were taken with a fisheye lens. The captured images were analyzed using the Gap Light Analyzer 2.0 software (Cary Institute of Ecosystem Studies, New York, USA). BHA was measured within a 20 x 20 m² quadrat in the center of each site.

The relative light intensity was measured to determine differences in light conditions in the natural habitats of C. japonicum. At the same locations where canopy openness was measured, photometric measurements were recorded from sunrise to sunset using quantum sensors (LI-190R, Apogee Instruments Inc., Logan, USA) and data loggers (MSTL, STA Corp., Anyang, Korea). The relative light intensity (RLI) of the diffused light within the stand was calculated using the measured light intensity using the same method as Sasaki (1983). To examine the light conditions in the forest, a scattergram was plotted with sunlight intensity outside the stand on the x-axis and light intensity in the stand measured at the same time on the y-axis.
The photosynthetic responses of *C. japonicum* leaves were investigated using fully matured leaves from four naturally growing *C. japonicum* measured from the different study sites.

A portable photosynthesis system (Li-6400XT, Li-Cor Inc., Lincoln, USA) with an LED light source (6400-02, Li-Cor Inc., Lincoln, USA) and a CO₂ injector system (6400-01, Li-Cor Inc., Lincoln, USA) was used to measure the photosynthetic responses to different levels of light intensity. To prevent measurement errors due to photoinhibition and dew, measurements were made within 3 h after sunrise. During measurements, airflow into the chamber was set to 500 μmol s⁻¹, CO₂ was set to 400 ppm and temperature to 25 ± 2 °C. Based on the photosynthetic response model of Farquhar et al. (1980) and Farquhar and Sharkey (1982), a light–response curve was created by the photosynthetic responses, and this was used to obtain apparent quantum yield (AQY), dark respiration (Rd), light compensation point (LCP), light saturation point (LSP), and maximum photosynthesis rate (P_{Nmax}). The interpretation of the light–response curve was based upon the method of Kim and Lee (2001a).

To measure photosynthetic responses to changes in CO₂ concentration, air flow into the chamber was set to 500 μmol s⁻¹, light intensity was set to 1000 μmol m⁻² s⁻¹, and temperature was set to 25 ± 2 °C. The CO₂ flowing into the chamber was set to various concentrations (50; 100; 150; 250; 350; 600; and 800 μmol mol⁻¹) and the photosynthetic rate was measured. Further, the CO₂ concentration in the mesophyll was calculated using the method of Farquhar and Sharkey.
Chlorophyll a, b, and a/b per leaf area (g·m⁻²), and the ratio of chlorophyll a/b were calculated using the equation below (Wellburn 1994).

Chlorophyll a = 12.47 A₆₆₅.₁ − 3.62 A₆₄₉.₁
Chlorophyll b = 25.06 A₆₄₉.₁ − 6.50 A₆₆₅.₁

To investigate the morphological changes of the leaves in response to light conditions, leaf mass per area (LMA) was obtained. In August 2018, mature leaves were collected from 10 C. japonicum plants from each site, and the area of each collected leaf was measured using a portable leaf area meter (Li-3000C, Li-Cor Inc., Lincoln, USA).

| Site | Altitude (m) | Date       | Temperature (°C) Stand in | Stand out | Relative humidity (%) Stand in | Stand out |
|------|-------------|------------|---------------------------|-----------|-------------------------------|-----------|
| PC   | 470         | Apr. 26    | 17.6 (± 4.7)              | 13.3 (± 5.6) | 47.4 (±16.1)                  | 55.1 (±24.5) |
|      |             | Jun. 12    | 16.9 (± 3.0)              | 20.6 (± 3.8) | 90.0 (±10.3)                  | 69.1 (±17.0) |
|      |             | Aug. 08    | 26.1 (± 1.9)              | 29.5 (± 3.9) | 88.9 (± 8.1)                  | 54.5 (±15.3) |
|      |             | Apr. 27    | 14.3 (± 3.6)              | 15.0 (± 5.6) | 56.7 (±14.0)                  | 67.1 (±22.8) |
|      |             | Jun. 15    | 13.6 (± 1.0)              | 16.6 (± 4.1) | 96.1 (± 3.5)                  | 79.8 (± 6.3) |
|      |             | Aug. 10    | 23.1 (± 1.5)              | 27.3 (± 4.7) | 93.5 (± 3.7)                  | 77.8 (± 17.9) |
|      |             | Apr. 12    | 13.3 (± 4.4)              | 14.7 (± 3.6) | 36.2 (± 8.4)                  | 36.0 (± 7.2) |
|      |             | Jun. 01    | 19.7 (± 3.3)              | 22.3 (± 4.3) | 72.7 (±13.3)                  | 62.0 (± 12.2) |
|      |             | Aug. 02    | 25.9 (± 2.1)              | 30.5 (± 2.6) | 81.1 (± 6.3)                  | 68.3 (± 10.3) |

Values are means ± SD (n = 3).

Table 1. Temperature and relative humidity in three sites (PC: Pocheon; CC: Chunchon; GY: Gwangyang) within the natural habitat of Cypripedium japonicum.

Results
Growing conditions of each site
Temperature and relative humidity
We measured micrometeorological conditions (temperature and relative humidity) in the different study sites (Table 1). Height above sea level for the study sites was 470 m for PC, 779 m for CC, and 466 m for GY. In April 2018, the average daily temperature was measured at each measurement location, and the average temperatures were 17.6 °C for PC, 14.3 °C for CC, and 13.3 °C for GY. The average temperature in August of the same year was 26.1 °C for PC, 23.1 °C for CC, and 25.9 °C for GY, showing a tendency to increase in the same direction as the change of seasons. Average daily relative humidity in the same locations and periods increased with the change of seasons in a similar direction to the average temperature, being significantly higher in June and August than in April across all study sites.

Canopy structure
The average canopy openness (%) in April 2018 was 22.7 ± 0.7 for PC, 33.9 ± 3.2 for CC, and 20.7 ± 1.7 for GY (Figure 3(a)). The difference between the highest (CC) and lowest (GY) average canopy openness was significant. The average canopy openness was significantly lower at all study sites in June compared to those in April. In August, canopy openness was similar to those in June, but PC (9.1 ± 0.2) was significantly more open than CC (7.4 ± 2.7) and GY (8.2 ± 1.1).

BHAs of the different study sites are presented in Table 2. In PC, Kalopanax septemlobus was dominant, once the measurements were completed, the leaves were dried in a dryer and weighed. The LMA (mg·cm⁻²) was calculated using the area and dry weight of each leaf.

Statistical analysis
One-way ANOVAs were used for statistical analyses using SPSS version 21.0 (IBM Inc., New York, USA). Levene’s test was used to assess for homogeneity of variance, and post hoc analyses were performed using Scheffe’s significant difference test and Dunnett’s T3 test (p = 0.05) to determine significant differences between study sites.

(1982) and A–Ci curve was created. Based on the A–Ci curve, the carboxylation efficiency (CE, μmol(CO₂)·mol⁻¹), photo-respiration rate (Rp, μmol (CO₂) m⁻² s⁻¹), CO₂ compensation point (CCP, μmol(CO₂)·mol⁻¹), CO₂ saturation point (CSP, μmol(CO₂)·mol⁻¹), and maximum photosynthesis rate (P₅₅₆₆₅, μmol (CO₂) m⁻² s⁻¹) were calculated. The interpretation of A–Ci curve was based on the method of Kim and Lee (2001a).

Photosynthetic responses were measured under actual light conditions using the same portable photosynthesis system. The quantum sensor (LI-190R, Li-Cor Inc., Lincoln, USA) attached to the leaf chamber of the portable photosynthesis system detected changes in light intensity in the natural habitat and maintained the same light intensity in the chamber. Using this method, the photosynthetic responses, stomatal conductance, and transpiration rate of C. japonicum were measured from sunrise to sunset. Water use efficiency (WUE) during photosynthesis was calculated using the ratio of photosynthetic rate (Pn): transpiration rate (Tr) (Pn/Tr) that were simultaneously measured using the portable photosynthesis system (Kim et al. 2002).

**Table 1.** Temperature and relative humidity in three sites (PC: Pocheon; CC: Chunchon; GY: Gwangyang) within the natural habitat of Cypripedium japonicum.
was dominant with the highest BHA (22.7 m²/a), followed by *Celtis sinensis* and *Morus bombycis*. In CC, *Carpinus cordata* was dominant with the highest BHA (11.3 m²/a), followed by *Acer pseudosieboldianum* and *Fraxinus rhynchophylla*. In GY, *Juglans mandshurica* was dominant with the highest BHA (22.7 m²/a), followed by *Celtis sinensis*, and *Acer pictum* subsp. *mono*.

**Light conditions**

RLIs of diffused light were calculated at the same locations where canopy openness was measured (Figure 3(b)). The average RLI (%) in April 2018 was 8.1 ± 1.1 for PC, 2.0 ± 0.5 for CC, and 1.1 ± 0.2 for GY, while the measurements in August were 7.6 ± 0.5 for PC, 2.5 ± 0.4 for CC, and 1.3 ± 0.3 for GY, which were remarkably lower compared to the measurements made in April.

Light quality was measured at the same locations where light intensity was measured, and the R/FR ratios within the natural habitats were compared to those measured in sunlight outside the stand (Table 3). The R/FR ratios of natural light remained at 1.2–1.3 throughout the investigation period. The R/FR ratios of sunflecks measured in PC, CC, and GY in April 2018 were 0.9–1.0, which was lower than sunlight. The R/FR ratios of diffused light were lower still at 0.6, 0.8, and 0.9. In June, the R/FR ratios of sunflecks at each study site decreased to 0.3–0.8, and those of diffused light decreased to 0.1–0.2. In August, the R/FR ratios of sunflecks at each study site were 0.4–0.9, which were similar to those in June. However, the R/FR ratio of diffused light in PC was higher (0.4) than those of CC (0.2) and GY (0.1).

**Chlorophyll content and LMA**

Differences in the chlorophyll content of *C. japonicum* in each natural habitat were analyzed (Table 4). Chlorophyll a, b, and a + b content (g.m⁻²) was highest in GY, followed by CC and PC. Chlorophyll a, b, and a + b content was similar between CC and GY, but chlorophyll a, b, and a + b content in PC was significantly lower than those of CC and GY. Chlorophyll a/b was highest in PC (3.9 ± 0.5), followed by CC

![Table 2. Mean breast height area (BHA, m²/ha⁻¹) of woody species in three sites (PC: Pocheon; CC: Chuncheon; GY: Gwangyang) within the natural habitat of *Cypripedium japonicum*.](image)

| Species                   | PC   | CC   | GY   | Total |
|---------------------------|------|------|------|-------|
| *Acer pictum* subsp. *mono* | 0.0  | 5.0  | 0.0  | 5.0   |
| *Acer pseudosieboldianum*  | 0.0  | 5.0  | 0.0  | 5.0   |
| *Acer trilobum*            | 0.0  | 5.0  | 0.0  | 5.0   |
| *Aralia elata*             | 0.0  | 5.0  | 0.0  | 5.0   |
| *Carpinus cordata*         | 0.0  | 5.0  | 0.0  | 5.0   |
| *Carpinus laxiflora*       | 0.0  | 5.0  | 0.0  | 5.0   |
| *Celtis sinensis*          | 0.0  | 5.0  | 0.0  | 5.0   |
| *Cornus controversa*       | 0.0  | 5.0  | 0.0  | 5.0   |
| *Fraxinus rhynchophylla*    | 0.0  | 5.0  | 0.0  | 5.0   |
| *Juglans mandshurica*      | 0.0  | 5.0  | 0.0  | 5.0   |
| *Kalopanax septemlobus*    | 0.0  | 5.0  | 0.0  | 5.0   |
| *Lindera obtusiloba*       | 0.0  | 5.0  | 0.0  | 5.0   |
| *Maackia amurensis*        | 0.0  | 5.0  | 0.0  | 5.0   |
| *Magnolia sieboldii*       | 0.0  | 5.0  | 0.0  | 5.0   |
| *Meliosma myriantha*       | 0.0  | 5.0  | 0.0  | 5.0   |
| *Morus bombycis*           | 0.0  | 5.0  | 0.0  | 5.0   |
| *Neobalanca japonica*      | 0.0  | 5.0  | 0.0  | 5.0   |
| *Philadelphus schrenkii*    | 0.0  | 5.0  | 0.0  | 5.0   |
| *Prunus padus*             | 0.0  | 5.0  | 0.0  | 5.0   |
| *Quercus variabilis*       | 0.0  | 5.0  | 0.0  | 5.0   |
| *Staphylea buma*           | 0.0  | 5.0  | 0.0  | 5.0   |
| *Styrax japonicus*         | 0.0  | 5.0  | 0.0  | 5.0   |
| *Styrax obassia*           | 0.0  | 5.0  | 0.0  | 5.0   |
| *Tilia manshurica*         | 0.0  | 5.0  | 0.0  | 5.0   |
| **Total**                  | 48.0 | 42.1 | 67.2 | 157.2 |

**Table 3. Red/far-red (R/FR) ratios in three sites (PC: Pocheon; CC: Chuncheon; GY: Gwangyang) within the natural habitat of *Cypripedium japonicum*.**

| Light type     | Site       | April     | June      | August    | Mean    |
|----------------|------------|-----------|-----------|-----------|---------|
| Sunlight       | PC         | 1.2 a     | 1.2 a     | 1.3 a     | 1.3 a   |
| Sunfleck PC    | 0.9 b      | 0.9 b     | 0.9 b     | 0.9 b     | 0.9 b   |
| Sunfleck CC    | 1.0 c      | 1.0 c     | 1.0 c     | 1.0 c     | 1.0 c   |
| Sunfleck GY    | 0.8 d      | 0.8 d     | 0.8 d     | 0.8 d     | 0.8 d   |
| Diffused light PC | 0.6 e | 0.6 e   | 0.6 e     | 0.6 e     | 0.6 e   |
| Sunfleck CC    | 1.0 f      | 1.0 f     | 1.0 f     | 1.0 f     | 1.0 f   |
| Sunfleck GY    | 0.9 g      | 0.9 g     | 0.9 g     | 0.9 g     | 0.9 g   |

It was compared how much the R/FR ratio of the sunfleck and diffused light decreased compared to sunlight. The R/FR ratio of diffused light was significantly reduced compared to that of sunlight and sunfleck.
(3.8 ± 0.1) and GY (3.3 ± 0.2), but differences among the sites were nonsignificant.

The LMA (mg cm⁻²) of *C. japonicum* was the highest in PC (4.2 ± 0.4), followed by CC (3.6 ± 0.6) and GY (3.1 ± 0.2).

### Photosynthetic characteristics

AQY, Rd, LCP, LSP, and PNmax calculated from the light–response curve describing the photosynthetic characteristics of *C. japonicum* (Figure 4(a)), are summarized in Table 5. The AQY (mmol(CO₂) mol⁻¹) of *C. japonicum* was 39.2 ± 1.9 in PC, 39.0 ± 7.7 in CC, and 53.5 ± 3.6 in GY. AQY was similar between PC and CC, but the AQY in GY was significantly lower and 53.5 ± 3.6 in GY. AQY was similar between PC (39.2 ± 1.9), CC (39.0 ± 7.7), and PC (39.2 ± 1.9) was significantly lower than the other two sites. The Rd (µmol(CO₂) m⁻² s⁻¹) was similar between sites (1.0 ± 0.0 in PC, 1.2 ± 0.4 in CC, and 1.1 ± 0.2 in GY). The LCPs (µmol m⁻² s⁻¹) was higher in habitats with greater number of sunflecks (Figure 5). When the observed diurnal variation was examined in terms of photosynthetic rate in relation to light intensity, the photosynthetic rate in PC was the highest, followed by CC, and GY (Figure 6(a)). Transpiration rate and stomatal aperture were also higher in habitats with greater number of sunflecks (Figure 6(b,c)). Examining the changes in WUE in relation to light intensity showed that WUE was the lowest at the LCP where photosynthetic rate changed into a positive value in all habitats, but WUE increased with increasing luminosity (Figure 6(d)). In addition, were 13.3 ± 5.4 in PC, 19.0 ± 3.7 in CC, and 29.0 ± 1.4 in GY, with LCP in GY being higher than that in PC and CC. The LSPs (µmol m⁻² s⁻¹) were 305.0 ± 13.5 in PC, 184.0 ± 8.6 in CC, and 225.0 ± 4.2 in GY, and differences among them were significant. The PNmax (µmol(CO₂) m⁻² s⁻¹) was highest in PC (3.5 ± 0.1), followed by CC (3.0 ± 0.5) and GY (2.4 ± 0.1).

The A–Ci curve in Figure 4(b) shows the photosynthetic rate measured at different CO₂ concentrations and the other parameters (Table 6) revealed that CE was the highest in PC, followed by CC and GY, and the differences were significant. While CCP and PNmax were also significantly different, the differences in Rp and CSP among the habitats were nonsignificant.

### Table 4. Contents of chlorophyll a, b, a + b, a/b ratio, and leaf mass per area (LMA) in three sites (PC: Pocheon; CC: Chuncheon; GY: Gwangyang) within the natural habitat of *Cypripedium japonicum*.

| Site | a (g/m²) | b (g/m²) | a + b (g/m²) | a/b | LMA (mg/cm²) |
|------|----------|----------|-------------|-----|--------------|
| PC   | 1.4 (±0.3) | 0.4 (±0.0) | 1.8 (±0.3) | 3.9 (±0.5) | 4.2 (±0.4) |
| CC   | 2.1 (±0.3) | 0.6 (±0.1) | 2.7 (±0.4) | 3.8 (±0.1) | 3.6 (±0.6) |
| GY   | 2.2 (±0.1) | 0.7 (±0.0) | 2.9 (±0.1) | 3.3 (±0.2) | 3.1 (±0.2) |

Chlorophyll contents and LMA are represented by different letters according to scheffe’s post hoc test (p = 0.05). Values are means ± SD (n = 10).

### Table 5. Photosynthesis characteristics of *Cypripedium japonicum* plants grown in three sites (PC: Pocheon; CC: Chuncheon; GY: Gwangyang).

| Site | AQY (mmol(CO₂) mol⁻¹) | Rd (µmol(CO₂) m⁻² s⁻¹) | LCP (µmol m⁻² s⁻¹) | LSP (µmol m⁻² s⁻¹) | PNmax (µmol(CO₂) m⁻² s⁻¹) |
|------|----------------------|------------------------|---------------------|---------------------|--------------------------|
| PC   | 39.2 (±1.9)          | 1.0 (±0.0)             | 13.3 (±5.4)         | 305.0 (±13.5)       | 3.5 (±0.1)               |
| CC   | 39.0 (±7.7)          | 1.2 (±0.4)             | 19.0 (±3.7)         | 184.0 (±8.6)        | 3.0 (±0.5)               |
| GY   | 53.5 (±3.6)          | 1.1 (±0.2)             | 29.0 (±1.4)         | 225.0 (±4.2)        | 2.4 (±0.1)               |

Net apparent quantum yield; AQY; dark respiration; Rd; light compensation point; LCP; light saturation point; LSP; maximum photosynthesis rate; PNmax; Photosynthesis characteristics are represented by different letters according to scheffe’s post hoc test (p = 0.05). Values are means ± SD (n = 4).
the highest WUE was reached at the LSP, but it gradually decreased with increasing light intensity above the LSP. Difference in WUE at the three habitats was not large in all ranges of light intensity.

**Discussion**

Temperatures in the natural habitat of *C. japonicum* were relatively lower than those measured outside the stand (Table 1). Temperatures in the natural habitat were significantly higher in August compared to April but decreased in June. Temperature increases were small compared to those recorded outside the stand. This could be due to the natural habitat being situated on the slopes of the valley and due to the influences of monsoons. In contrast to findings reported by Pi et al. (2015), temperature differences between study sites were not significant. Relative humidity was 73% or higher across all study sites. These findings indicate that orchids, such as *C. japonicum*, are distributed in environments where sufficient air humidity is maintained, and is consistent with the findings of Cribb (1997) who stated that *C. japonicum* usually grows in the shade in the forest. *C. japonicum* are found on northeast facing slopes in both South Korea (Pi et al. 2015) and Japan (Nagamatus 2011). In general, northeast facing slopes in the forest have high soil moisture and air humidity (KFRI 2013). Possibly, high air humidity is a result of the direction of the slope. The vegetation in the natural habitats of *C. japonicum* was dominated by mountain stream vegetation such as *F. rhynchophylla*, *A. pictum*, *C. controversa*, and *J. mandshurica* (Table 2). These species indicate that *C. japonicum* are naturally distributed around valleys, where a high average annual humidity is maintained at 73% or higher.

In general, plants grown in low light conditions have a higher total chlorophyll content (Kim and Lee 2001a) to maximize photoreception capacity. This proved true in the present study where the Chlorophyll

**Table 6. Photosynthesis characteristics of Cypripedium japonicum calculated from the A–Ci curve in Figure 4 (PC: Pocheon; CC: Chuncheon; GY: Gwangyang).**

| Site | CE (umol(CO₂) mol⁻¹) | R₂ (umol(CO₂) m⁻² s⁻¹) | CCP (umol(CO₂) mol⁻¹) | CSP (umol(CO₂) mol⁻¹) | Pₙₜₘₐₓ (umol(CO₂) m⁻² s⁻¹) |
|------|----------------------|------------------------|----------------------|----------------------|--------------------------|
| PC   | 0.015ₚ ± 0.001       | 0.9ₚ ± 0.2             | 60.1ₚ ± 4.1          | 352.8ₚ ± 3.8         | 3.9ₚ ± 0.1               |
| CC   | 0.013ₚ ± 0.002       | 1.3ₚ ± 0.1             | 85.4ₚ ± 7.8          | 322.6ₚ ± 4.5         | 3.4ₚ ± 0.4               |
| GY   | 0.010ₚ ± 0.003       | 1.4ₚ ± 0.3             | 111.9ₚ ± 9.7         | 362.5ₚ ± 5.7         | 2.4ₚ ± 0.5               |

Carboxylation efficiency; CE: photo-respiration rate; R₂: CO₂ compensation point; CCP: CO₂ saturation point; CSP: maximum photosynthesis rate; Pₙₜₘₐₓ. Photosynthesis characteristics are represented by different letters according to scheffe’s post hoc test (p = 0.05). Values are means ± SD (n = 4).

**Figure 5.** Changes in the photosynthetic response of Cypripedium japonicum in three the natural habitats in August 2018. PC: Pocheon; CC: Chuncheon; GY: Gwangyang; PPFD: photosynthetic photon flux density; gray line: light intensity outside the stand; dark gray line: light intensity inside the stand; ×: mark: photosynthetic rate.
content in *C. japonicum* was higher in sites where the relative light was lower (Figure 3(b) and Table 4). Under low light conditions, chlorophyll b, which makes up light-harvesting complexes, increases more than chlorophyll a, which is mainly bound to the reaction center, as an adaptation to collect more light. Because of this, decreased chlorophyll a/b indicates leaf shading (Kim et al. 2008; Lee et al. 2019). Chlorophyll a/b decreases in plants acclimatize to low light conditions. Similarly, chlorophyll a/b of *C. japonicum* decreased when the shading level was high, enabling shade tolerance (Terashima and Hikosaka 1995). LMA is leaf dry mass per unit of leaf area and is widely used in plant ecology and other fields to indicate leaf thickness, mesophyll density, and the relationship between plant growth, carbon gain, and light absorption (Kattge et al. 2011; Cheng et al. 2014). In trees, the relationship between LMA and level of shading greatly varies between species (Loach 1970), but the relationship generally becomes stronger as shading increases (Song et al. 2014; Lee et al. 2019). However, the LMA of *C. japonicum* was lowest in GY, despite having higher levels of shading compared to PC and CC. The LMA of *C. japonicum* in GY, was the lowest in areas of highest shading (Table 4), and in general, the lack of nitrogen in leaves could be considered first as the cause. Pi et al. (2015) also stated that the leaf area of *C. japonicum* was lower in rocky areas and in the case of GY, they reported that the growth may be hindered through reduced soil availability. In another study, it was concluded that, if the activity of the photosynthetic system and photosynthetic capacity are reduced solely due to the deterioration of nutritional conditions, only total chlorophyll content will be reduced without changes in chlorophyll a/b (Terashima and Evans 1988; Hikosaka 1996). However, in the present study, in case of *C. japonicum* in GY, the chlorophyll a + b content increased while chlorophyll a/b decreased slightly, indicating that this cannot be readily interpreted as being caused only by the deterioration of nutritional conditions.

The canopy openness of the natural habitats of *C. japonicum* was 20.7–33.9% in April when the canopy was relatively open, but then gradually decreased to less than 10% as the canopy closed (Figure 3(a)). In terms of light intensity in the stand, the upper part of the canopy receives a lot of natural light, but light intensity decreases significantly as light passes through the canopy (Inada 1980; Smith 1995). The RLI on the forest floor is affected by spatial variables and may vary depending on canopy openness and density (Gu...
et al. 2002; Baudry et al. 2014). Canopy openness is positively correlated with relative light intensity (Kim et al. 2019), which was found to decrease with canopy closure in the natural habitats of C. japonicum and the decrease was larger in CC and GY where canopy openness was less than PC (Figure 3(b)). As a result of the leaf layer of the canopy selectively absorbing and using red light for photosynthesis, the light reaching the lower part of the canopy has a reduced R/FR ratio, affecting plant photomorphogenesis (Ammeter 2003). Even in the three habitats of C. japonicum, the R/FR ratio of diffused light was greatly decreased with the absorption in the red wavelength region by the canopy while the R/FR ratios of sunflecks slightly decreased compared to that of sunlight, which is similar to earlier reports (Inada 1980; Smith 1995; Kim et al. 2019; Table 3). During the day, light in the forest is mostly diffused light and sunflecks reaching the forest floor through canopy gaps (about 10%) (Chazdon and Pearcy 1991). Plants growing in the forest floor, where the amount of sunlight is likely to be limited, mostly rely on sunflecks for their photosynthetic production. Sunflecks contribute to 20–90% of materials synthesized in plants growing on the forest floor (Chazdon and Pearcy 1991). Sunflecks also showed a series of correlations with canopy density, and light intensity observed in the three habitats in August 2018 showed even distribution of light throughout the day in PC while CC and GY had only a short light exposure in the morning and afternoon, respectively (Figure 5). Although the weather was not clear in PC when the light intensity was measured, sunflecks were frequently observed while the frequency of sunflecks was lower in GY despite the weather being clear with minimal clouds. In the case of CC, the sunlight was lower than the other two habitats due to the large amount of clouds but judging by the similar lesser canopy openness of GY, the frequency of sunflecks is assumed to be low even in clear weather. The average daily photosynthetic activity (μmol m⁻² s⁻¹) of C. japonicum was 3.43 (maximum 6.39) in PC, but it was lower in CC and GY with 0.34 (maximum 2.27), and 0.65 (maximum 3.54), respectively (Figure 5). Due to changes in light intensity, habitats with higher frequency of sunflecks showed faster photosynthetic rate at higher light intensity (Figure 6(a)). It is our understanding that the higher the photosynthetic rate, the higher the production of assimilation products resulting in decrease of CO₂ concentration in the mesophyll, leading to an increase in stomatal aperture and higher transpiration (Figure 6(c,d)).

Study of the photosynthetic response of C. japonicum to changes in light intensity revealed that the AQY and LCP increased with the decrease of the relative light intensity, but the LSP and Pₐmax decreased (Table 5). AQY indicates photosynthetic capacity under low light conditions (0-100 μmol·m⁻²·s⁻¹), and it reflects the activation of the photosystem I that converts light energy into chemical energy (Evans 1987; Kim and Lee 2001a). Plants grown under low light conditions have a higher AQY by actively investing leaf nitrogen in chlorophyll production to optimize photosynthesis (Kim and Lee 2001a). C. japonicum had a higher AQY under high shade conditions. Plants with greater shade tolerance have LCPs of 5–10 μmol·m⁻²·s⁻¹, and LSPs of 100–200 μmol·m⁻²·s⁻¹ (Larcher 1995). The LCP and LSP of C. japonicum exceeded the range of shade plants, and differences in the Pₐmax in each study site showed similar trends to differences in canopy openness and RLI. A–C₁ curve, which is a photosynthetic response (A) curve to CO₂ concentration in the mesophyll, is commonly used to evaluate the capability of carbon fixation (Farquhar and Sharkey 1982; Kim and Lee 2001a). In the A–C₁ curve, the C₁ values from CO₂ concentration in the air to CO₂ saturation point are in the range where photosynthetic rate is determined by the rate of electron transport in photosystem II and photosystem I (Kim and Lee 2001a). An examination of photosynthetic rate at the CO₂ saturation point indicated that the electron transport rate decreased because the CO₂ concentration was low in habitats with low light (Figure 4(b)). The Pₐmax, which shows the rate of rephosphorylation of the carbon fixation, also decreased under low light conditions indicating that the rate of rephosphorylation decreased with an increase of CO₂ concentration (Table 6). Plants with high adaptability to the conditions of increased CO₂ concentration have high nitrogen use efficiency in photosynthesis, and do not show photoinhibition (Nakamura et al. 2011; Lee et al. 2014). Carboxylation efficiency can be considered as an indicator of rubisco content, and it was determined that the decreased light requirement caused by decreased carboxylation efficiency under low light conditions affected the activity of Rubisco. On the other hand, photorespiration, which is antagonistic to carboxylation efficiency, increased under low light conditions. Photorespiration occurs due to the characteristic of Rubisco to catalyze oxygenation reactions. This increase in photorespiration resulted in a decreased CO₂ uptake rate due to stomatal closure while the oxygen partial pressure in mesophyll relatively increased, resulting in the relative promotion of binding of Rubisco and oxygen (Oh et al. 2005). Plants growing in low light conditions increase the amount of Light-harvesting complex II (LHC II) in photosystem II, which binds to chlorophyll b and absorbs light and converts it into chemical energy (Brestic et al. 2014). However, the overall content of the protein that carries out electron transfer in the photosystem II and the content of Rubisco, a carbon-fixing enzyme of the dark reaction decrease (Evans 1987; Terashima and Hikosaka 1995). Therefore, the leaves adapted to the low light conditions show low photosynthesis at the light intensity above the light saturation point because the contents of electron transport system protein and rubisco are low. In summary, it appears that the activity of photosystem and carbon fixation of C. japonicum decreased by increased stress due to the lack of light in an environment with insufficient light, resulting in reduced photosynthetic capacity.

The results of the quantitative evaluation of light conditions in the three natural habitats of C. japonicum showed that the relative light intensity of
diffused light and the frequency of sunflecks with a high R/FR ratio decreased with decrease in canopy openness. In other words, increase in canopy density not only reduced the amount of light but also the quality of light required for plant growth. Because plants growing under these low light conditions cannot have high photosynthetic rates, high photosynthetic rates will not be a great advantage in acquiring energy required to grow and sustain life. Accordingly, reducing energy consumption by maintaining low photosynthetic capacity is more effective. Morphological and physiological characteristics change by the growth conditions even within the same species, and it appears that *C. japonicum* also shows adaptive responses to environmental stresses such as insufficient light. Biomass refers to plant biomass formed by storing solar energy as chemical energy through photosynthetic responses (Shin et al. 2011), and the production of biomass also appears to decrease due to the reduction in the amount of photosynthesis of *C. japonicum* under reduced relative light intensity and frequency of sunflecks due to crown closure. That is, crown closure may not only reduce the vigor of the plants in the year but also adversely affect the propagation in the following year by reducing the production of biomass needed for propagation (Willems et al. 2001; Horvitz et al. 2010). Pi et al. (2015) analyzed the relationship between stand and environmental factors and reported that the distribution of *C. japonicum* was higher in areas where canopy openness was higher. The natural habitats of the genus *Cypripedium* in the Korean Peninsula are dominated by deciduous broad-leaved trees. *C. japonicum* rapidly grows in early spring, and produces flowers and fruits before the last part of May when the coverage in the upper canopy increases to 70–80% (Park and Kim 1995). As such, light intensity can be considered a leading factor that limits the growth of *C. japonicum*, given that the numbers of *C. japonicum* increased after improving light conditions by removing the silver grass support (Takayuki 2014). However, caution should be exercised since the excessive opening of the canopy can cause photo-inhibition (Kim and Lee 2001b), due to high light intensity, and dry soil that can adversely affect the growth of *C. japonicum* (KNA 2017). The local conservation of *C. japonicum* is mostly carried out by installing protective facilities such as fences. However, this method has shortcomings such as short-lived effects and costly installation and management of the facility. For the long-term and effective conservation of *C. japonicum*, reasonable monitoring and survey of the natural habitats, as well as various studies for conservation in other areas, are necessary. Lighting is crucial for the photosynthesis and growth of plants. However, this study is limited in that only light was considered while plants grow through interactions with many environmental factors such as light, soil, and micrometeorological conditions. Conservation of *Cypripedium japonicum* should be carefully approached, and future studies can have better results by considering other environmental factors than light. The findings of our study, which analyzed the physiological characteristics of *C. japonicum* according to the light conditions in natural habitats, are expected to facilitate the local conservation of endangered *C. japonicum* and play an important role in conservation strategies in other areas.

**Conclusion**

An examination of light conditions in the habitats of *C. japonicum* showed differences in canopy density among the three habitats, and the relative light intensity and R/FR ratio of light quality observed in the forest floor showed a similar tendency to canopy openness. The LMA was higher in good light conditions due to greater canopy openness, and chlorophyll content was also significantly lower. The average daily photosynthetic activity was higher where the frequency of sunflecks was relatively high, and stomatal aperture and the transpiration rate were also higher. The activity of photosystem and carbon fixation was compared among the three habitats through light–response curve and A–Ci curve, and photosynthetic capacity was found to decrease due to increased stress caused by insufficient light in a low light environment. The growth and physiological characteristics of the plants growing in different habitats were closely related to the light conditions in the stand, and improvements of light conditions by controlling canopy density may influence their propagation. This study identified differences in the physiological characteristics of *C. japonicum* with differences in environmental conditions, and we believe that the findings have important implications in the prediction of population dynamics and the long-term conservation and restoration strategies of *C. japonicum*.

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**References**

Ammer C. 2003. Growth and biomass partitioning of *Fagus sylvatica* L. and *Quercus robur* L. seedlings in response to shading and small changes in the R/FR-ratio of radiation. Ann Sci. 60(2):163–171.

Bae KH. 2012. In vitro propagation of three rare and critically endangered *Cypripedium* species. [Ph.D. dissertation]. Korea: University of Gangwon.

Baudry O, Charmetant C, Collet C, Ponette Q. 2014. Estimating light climate in forest with the convex densiometer: operator effect, geometry and relation to diffuse light. Eur J Forest Res. 133(1):101–110.

Brestic M, Zivcak M. 2013. PSI1 fluorescence techniques for measurement of drought and high temperature stress signal in crop plants: protocols and applications. In: Gyana Ranjan Rout and Anath Bandhu Das (Eds.), Molecular stress physiology of plants. India: Springer; p. 87–131.
Brestic M, Zivcak M, Olsovska K, Shao HB, Kalaji HM, Allakhverdiev SI. 2014. Reduced glutamine synthetase activity plays a role in control of photosynthetic responses to high light in barley leaves. Plant Physiol Biochem. 81:74–83.

Chazdon RL, Peary RW. 1991. The importance of sunflecks for forest understory plants. Bioscience. 41(11):760–766.

Cheng T, Rizzarelli B, Sánchez-Azofeifa AG, Férét JB, Jaccoumeaud S, Ustín SL. 2014. Deriving leaf mass per area (LMA) from foliar reflectance across a variety of plant species using continuous wavelet analysis. ISPRS Int J Geoinf. 87:28–38.

Cho YC, Kim HG, Koo BY, Shin JK. 2019. Dynamics and viability analysis of transplanted and natural lady's slipper (Cypripedium japonicum) populations under habitat management in South Korea. Restor Ecol. 27(1):23–30.

Chung JM, Park KW, Park CS, Lee SH, Chung MG, Chung MY. 2009. Contrasting levels of genetic diversity between the historically rare orchid Cypripedium japonicum and the historically common orchid Cypripedium macranthos in South Korea. Bot J Linn Soc. 160(2):119–129.

Coates F, Lunt ID, Tremlay RL. 2006. Effects of disturbance on population dynamics of the threatened orchid Prasophyllum correctum D.L. Jones and implications for grassland management in south-eastern Australia. Biol Conserv. 129(1):59–69.

Cribb P. 1997. The genus cypripedium. Portland (OR): Timber Press.

De Jong TJ, Goosen-de Roo L, Klinkhamer PGL. 1998. Is the size of canopy crown a cause of differences in the quantum yield of net photosynthesis between sun and shade leaves? Funct Plant Biol. 15(4):69–77.

Farquhar GD, von Caemmerer S, Berry JA. 1980. A biochemical model of photosynthetic CO2 assimilation in leaves of C3 species. Planta. 149(1):78–90.

Farquhar GD, Sharkey TD. 1982. Stomatal conductance and photosynthesis. Annu Rev Plant Physiol. 33(1):317–345.

Ge L, Baldocchi D, Verma SB, Black TA, Falge E, Dwyer PW. 2000. Advantages of diffuse radiation for terrestrial ecosystem productivity. J Geophys Res Atmos. 107(D6):4050.

Hikosaka K. 1996. Effects of leaf age, nutrient nitrogen and photon flux density on the organization of the photosynthetic apparatus in leaves of a vine (Ipomoea tricolor Cav.) grown horizontally to avoid mutual shading of leaves. Planta. 198(1):144–150.

Hiscox JD, Israelstam GF. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can J Bot. 57(12):1332–1334.

Horvitz CC, Ehrlen J, Matlagn D. 2010. Context-dependent pollinator limitation in stochastic environments: can increased seed set overpower the cost of reproduction in an understory herb? J Ecol. 98(2):268–278.

Inada K. 1980. Spectral absorption property of pigments in living leaves and its contribution to photosynthesis. Jpn J Crop Sci. 49(2):286–294.

Janczuková P, Wotavková K, Schödelbauerová I, Jersáková J, Kindlmann P. 2006. Relative effects of management and environmental conditions on performance and survival of populations of a terrestrial orchid, Dactylorhiza majalis. Biol Conserv. 129(1):40–49.

Katge J, Díaz S, Lavorel S, Prentice IC, Leadley P, Börsch G, Garnier E, Westoby M, Reich PB, Wright JI, et al. 2011. TRY—a global database of plant traits. Glob Chang Biol. 17(9):2905–2935.

KFRI (Korea Forest Research Institute). 2013. Composition and management of oak forests in South Korea. KFRI Technical Report. 73:1–46.

Kim DH, Son SW, Suh GU, Jung JY, Lee JC, Kim PG. 2014. Analysis of the light condition in the forest stand (1). J Agric Life Sci. 53(5):75–82.

Kim JS, Kim HT, Son SW, Kim JH. 2015. Molecular identification of endangered Korean lady's slipper orchid (Cypripedium, Orchidaceae) and related taxa. Botany. 93(9):603–610.

Kim PG, Kim SH, Lee SM, Cho JH, Lee EJ. 2002. Photosynthetic responses of Populus alba > P. glandulosa in adaptation to 'Kimpo' waste landfills. J Korean Soc Sci. 91(1):79–87.

Kim PG, Lee EJ. 2001a. Ecophysiology of photosynthesis 1: effects of light intensity and intercellular CO2 pressure on photosynthesis. Korean J Agric Meteorol. 3(2):126–133.

Kim PG, Lee EJ. 2001b. Ecophysiology of photosynthesis 2: adaptation of the photosynthetic apparatus to changing environment. Korean J Agric Meteorol. 3(3):171–176.

Kim SH, Saung JH, Kim YK, Kim PG. 2008. Photosynthetic responses of four oak species to changes in light environment. Korean J Agric Meteorol. 10(4):141–148.

KNA (Korea National Arboretum). 2008. Rare plants data book. In Korea. Pochon: Korea National Arboretum.

KNA (Korea National Arboretum). 2017. Population dynamics of Korean lady's slipper (Cypripedium japonicum) in Gwangneung Forest Biosphere Reserve (GFBR) in South Korea. Seoul: Korea National Arboretum.

Larcher W. 1995. Physiological plant ecology. Germany (BE): Springer.

Lee BH, Han HK, Kwon HJ, Ahn HE. 2015. Diversity of endophytic fungi isolated from roots of Cypripedium japonicum and C. macranthos in Korea. Kor J Mycol. 43(1):20–25.

Lee DH, Kim SD, Kim HM, Moon AR, Kim SY, Park BB, Son SW. 2021. Stage structure and population persistence of Cypripedium japonicum Thunb., a rare and endangered plants. Korean J Environ Ecol. 35(5):548–557.

Lee KC, Han SK, Kwon YH, Jeon SR, Lee CW, Seo DJ, Park WG. 2019. Effects of shading treatments on growth and physiological characteristics of Aruncus dioicus var. kamtschaticus (Maxim.) H. Hara seedling. JKMCS. 27(1):30–37.

Lee S, Oh CY, Han SH, Kim KW, Kim PG. 2014. Photosynthetic responses of Populus alba × glandulosa to elevated CO2 concentration and air temperature. Korean J Agric Meteorol. 16(1):22–28.

Loach K. 1970. Shade tolerance in tree seedlings II. Growth analysis of plants raised under artificial shade. New Phytol. 69(2):273–286.

Nagamatsu D. 2011. The present condition of Cypripedium japonicum Thunb. (Orchidaceae) population in Tottori Prefecture. Nat Hist Res San’in. 6:9–15.

Nakamura I, Onoda Y, Matsushima N, Yokoyama J, Kawata M, Hikosaka K. 2015. Phenotypic and genetic differences in a perennial herb across a natural gradient of CO2 concentration. Oecologia. 165(3):809–818.

Nam JW, Ku JJ, Choi JJ, Shin YM, Park SY, Kim MH, Pee JH, Son SW, Kim IK. 2014. The insect pollinators of Cypripedium japonicum Thunb., the endangered orchid in South Korea. In: Conference Proceedings and Presentations; p. 75.

Oh CY, Han SH, Kim YY, Lee JG. 2005. Changes of drought tolerance and photosynthetic characteristics of Populus davidiana dode according to PEG concentration. Korean J Agric Meteorol. 7(4):296–302.

Park KW, Kim SS. 1995. Studies on the wild Cypripedium japonicum in Korea (I); especially on the growth environment in natural forests. J Korean Soc Sci. 51:64–73.

Pi JH, Jung JY, Park JG, Yang HH, Kim EH, Suh GU, Lee CH, KNA (Korea National Arboretum) 2008. Rare plants data book. In Korea. Pochon: Korea National Arboretum.

Pi JH, Jung JY, Park JG, Yang HH, Kim EH, Suh GU, Lee CH, KNA (Korea National Arboretum) 2008. Rare plants data book. In Korea. Pochon: Korea National Arboretum.

P. glandulosa in adaptation to 'Kimpo' waste landfills. J Korean Soc Sci. 91(1):79–87.

Phylogenet. 43(7):4050.
Shin KS, Kim CH, Lee SE, Yoon YM. 2011. Biochemical methane potential of agricultural waste biomass. Korean J Soil Sci Fert. 44(5):903–915.

Sim MY, Youn JY, Chung JM, Lee BC, Koo CD, Eom AH. 2010. Characteristics of orchid mycorrhizal fungi from roots of Cypripedium japonicum and C. macranthum. Kor J Mycol. 38(1):1–4.

Smith H. 1995. Physiological and ecological function within the phytochrome family. Annu Rev Plant Physiol Plant Mol Biol. 46(1):289–315.

Song KS, Jeon KS, Yoon JH, Kim CH, Park YB, Kim JJ. 2014. Characteristics of growth and root development of Peucedanum japonicum seedling by shading rate and container size. Korean J Medicinal Crop Sci. 22(5):384–390.

Sun HQ, Cheng J, Zhang FM, Luo YB, Ge S. 2009. Reproductive success of non-rewarding Cypripedium japonicum benefits from low spatial dispersion pattern and asynchronous flowering. Ann Bot. 103(8):1227–1237.

Swarts ND, Dixon KW. 2009. Terrestrial orchid conservation in the age of extinction. Ann Bot. 104(3):543–556.

Takayuki B, Itoko K, Itse Y, Hanako Y, Gorda Y, Shoji Y, Takahashi H, Naohhito S, Murayama S. 2014. Attention points for habitat restoration of endangered species. Japan: Samrim Res Inst.

Terashima I, Evans JR. 1988. Effects of light and nitrogen nutrition on the organization of the photosynthetic apparatus in spinach. Plant Cell Physiol. 29:143–155.

Terashima I, Hikosaka K. 1995. Comparative ecophysiology of leaf and canopy photosynthesis. Plant Cell Environ. 18(10):1111–1128.

Tian HZ, Han LX, Zhang JL, Li XL, Kawahara T, Yukawa T, López-Pujol J, Kumar P, Chung MG, Chung MY. 2018. Genetic diversity in the endangered terrestrial orchid Cypripedium japonicum in East Asia: Insights into population history and implications for conservation. Sci Rep. 8(1):1–13.

Wellburn AR. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol. 144(3):307–313.

Willems JH, Balounová Z, Kindlmann P. 2001. The effect of experimental shading on seed production and plant survival of Spiranes spiralis (Orchidaceae). Lindleyana. 16(1):31–37.