# Leaf traits and photosynthetic characteristics of endangered *Sinopodophyllum hexandrum* (Royle) Ying under different light regimes in Southeastern Tibet Plateau

Q.Q. GUO[*,**], H.E. LI[*], C. GAO[*], and R. YANG[*,+]

*Institute for Forest Resources & Environment of Guizhou/College of Forestry, College of Agriculture, Guizhou University, 550025 Guiyang, Guizhou, China[*]*
*Research Institute of Tibet Plateau Ecology, Tibet Agricultural and Animal Husbandry College, 860000 Nyingchi, Tibet, China[**]*

## Abstract

*Sinopodophyllum hexandrum* (Royle) Ying, a medicinal plant, has been endangered due to alpine habitat and overuse at Southeastern Tibet Plateau. This study aimed to examine the phenotypic traits and photosynthesis of *S. hexandrum*, including their relationship with main environmental factors, under low light (LL), moderate light (ML), and high light (HL) regimes. Low specific leaf mass was observed under LL, but the stomatal density and apparent quantum yield were the highest under this regime. The phenotypic traits, except for stomatal density and net photosynthetic rate, were the most notable under ML. The leaf area, stomatal density, apparent quantum yield, dark respiration, and stomatal conductance showed the smallest values under HL. Air temperature, photosynthetically active radiation, and air vapour pressure deficit significantly affected the stomatal characteristics and photosynthesis of *S. hexandrum* individuals. Our results suggested that a moderately shaded habitat could promote leaf development and the photosynthetic ability of *S. hexandrum*.

## Introduction

Plants, which are immovable organisms, grow under diversified light regimes to accumulate organic carbon through photosynthesis (Nadeem *et al*. 2015, Campos *et al*. 2016). In shrub communities, herbs are often placed below shrubs for intensive shading from sun exposure in a small clearing (Ou *et al*. 2015). Plants under different light intensities commonly exhibit morphological or physiological changes, especially in terms of the structure and function of leaves (Rodríguez-López *et al*. 2014, La Rocca *et al*. 2015). This phenomenon is the phenotypic trait for a given habitat, and is also a long-term-induced response of plants under a specific environment. Leaf phenotype is often regarded as an important way for plants to deal with environmental heterogeneity; this phenotype also indicates ecological adaptability (Santiago and Wright 2007, Guo *et al*. 2016). Thus, leaf traits are being explored at present to gain insight into the adaptability of plants to environmental stress.

Several parameters of leaf traits and photosynthesis, such as leaf area and perimeter (LA and LP), fresh leaf thickness (LT), chlorophyll (Chl) content, specific leaf mass (SLM), stomatal size and density ($S_s$ and $S_d$), net photosynthetic rate ($P_N$), and intrinsic water-use efficiency ($WUE_i$), can be used as representative indexes to evaluate the plant adaptation for sunny to shady environments (Givnish 1988). High LA and Chl result in large carbon assimilation and SLM under certain PAR. Stomatal activity can regulate the flux of $CO_2$ and $H_2O$ through the leaf surface. The negative correlation between $S_d$ and $S_s$ directly affects the stomatal conductance ($g_s$) and the maximum net photosynthetic rate ($P_{Nmax}$) (Camargo *et al*. 2011). Simultaneously, the effects of leaf traits and environmental factors on plant photosynthesis are also evident under different light regimes (Chapin *et al*. 1987). *Sinopodophyllum hexandrum* (Royle) Ying, Berberidaceae, is a perennial wild herb plant at the Southeastern Tibet

Plateau. This plant exhibits effective anticancer function (Liu *et al.* 2015). Its secondary metabolite is highly effective in curing malignant tumours, rheumatism, and gynecopathy (Kumari *et al.* 2014). Consequently, wild types of *S. hexandrum* are artificially dug randomly because *S. hexandrum* has been classified as an endangered species since 1987 and recorded in the Chinese Plant Red Book (Fu and Chin 1992). Given the low nature reproduction rate (Ma *et al.* 1997), genetic diversity (Xiao *et al.* 2006), and habitat fragmentation (Guo *et al.* 2012), *S. hexandrum* has been identified as a first-grade municipal endangered plant of Tibet in 2005 and 2009 (Lu *et al.* 2011). Therefore, the protection of existing resources and promotion of population recovery are current urgent tasks. Ma and Hu (1996) reported that fewer *S. hexandrum* plants grew in the open grassland with considerable under shrub shade at the Shangri-la region of China. Intense radiation can induce dark spots on the leaf surface at high altitude localities. No reference on effects of light intensity on physiological properties is available for *S. hexandrum*. Thus, the present work aimed to determine the leaf traits and photosynthetic characteristics of *S. hexandrum* under different light regimes. The present results can aid the identification of suitable methods for the recovery of wild populations of *S. hexandrum* and for guided artificial planting.

## Materials and methods

**Experimental site and materials**: This study was conducted at the Butterfly Valley (29°28'16"N, 94°22'41"E) at an altitude of approximately 2,996 m. This area is located in Bujiu Town, Nyingchi County, Southeast Tibet of China. The study area is a native alpine brush at the lower part of slope, which is the typical habitat of *S. hexandrum*. The mean annual temperature is 4.72°C, and the monthly maximum (July) and minimum (January) mean temperatures are 13.15°C and –4.82°C, respectively. The annual mean air relative humidity ($RH_{air}$) and sunshine duration are 75.3% and 1,150 h, respectively. The mean annual rainfall is approximately 650 mm, which is mainly concentrated from May to September. The area also experiences a severe dry season (≤80 mm per month) from October to April. The soil in the study area is a mountain brown with some stones, has medium fertility, and pH 4.2–4.5. The dominating shrub and herb species are *Rosa multiflora*, *Berberis julianae*, *Cotoneaster microphyllus*, *Hypericum hookerianum*, *S. hexandrum*, *Anemone rivularis*, *Potentilla fulgens*, and *Pteridium aquilinum*.

Twenty well-grown adult plants of *S. hexandrum* were selected as experimental objects under different light regimes. The average size and the detailed habitats are listed in Table 1. We measured the leaf traits and photosynthetic abilities of these samples. Because of the scarcity of wild *S. hexandrum* individuals, the $P_N$-PAR response curves and diurnal variations in photosynthesis of labelled leaves were determined first during sunny days from 12 July to 15–16 July, 2015. Second, the leaf traits were measured on 17–19 July, 2015. Each index was determined thrice during the course of experiments. Results are presented as means ± standard deviations.

**Leaf traits and stomatal characteristics**: LA, LP, LT, Chl, SLM, and stomatal characteristics ($S_s$ and $S_d$) were measured in 20 unbroken leaves of each labelled plant. LA, LP, and LT were measured using an LA meter (*AM-300*, *ADC*, UK) and a digital Vernier calliper (*EM-KC150*, *Elecall*, Shanghai, China). The Chl content was determined by the SPAD value (*SPAD-502*, *Konica*, Japan) and correlation equation [$y = 0.236e^{0.0588x}$ ($r^2 = 0.85$), where *x* indicates the *SPAD-502* value and *y* indicates the Chl content] (Uddling *et al.* 2007). Clear nail polish impressions (three per leaf) from the lower and upper leaf surfaces were obtained to determine the $S_s$ and $S_d$ from a sample with 20 stomata per leaf under a fluorescence microscope (*Eclipse 80i*, *Nikon*, Japan) using a magnification of 400× and 1,000×, respectively (Aasamaa and Sõber 2011). SLM was measured in accordance with the LM/LA ratio.

**$P_N$-PAR response curves**: The labelled leaves from *S. hexandrum* individuals were measured under uniform conditions [$20 \pm 0.5$°C, $60 \pm 5$% RH, and $380 \pm 5 \mu mol(CO_2)$ $mol^{-1}$] between 9:30 and 11:00 h. Responses to PAR were measured at the PAR range of 0–1,600 μmol(photon) $m^{-2}$ $s^{-1}$, whilst keeping the air flow of the photosynthesis measurement system (*Li-6400*, *Li-Cor*, Lincoln, USA) constant at 500 μmol $s^{-1}$. Data were automatically recorded using *Li-6400*.

Light-compensation point ($L_{CP}$) and saturation point ($L_{SP}$) of *S. hexandrum* were the PAR values when the values of $P_N$ were zero and maximum value, respectively. Linear regressions of PAR and $P_N$ over the range of 0–250 μmol(photon) $m^{-2}$ $s^{-1}$ were used to determine $L_{CP}$ and dark respiration ($R_D$). $L_{CP}$ was PAR value when photosynthetic activity balanced respiratory activity, and $R_D$ was the $P_N$ value when PAR was zero. Apparent quantum yield (α) was calculated as the slope of regression line. These curves were fitted using the following nonrectangular hyperbolic equation (Thornley 1976):

$$P_N = \frac{\alpha\,PAR + P_{Nmax} - \sqrt{(\alpha\,PAR + P_{Nmax})^2 - 4\alpha\,PAR\,P_{Nmax}}}{2\,k} - R_D$$

where *k* is the convexity factor (Farquhar and Sharkey 1982). Maximum net photosynthetic rate ($P_{Nmax}$) and $L_{SP}$ were estimated.

**Diurnal variation of gas exchange**: The diurnal variation in photosynthesis of *S. hexandrum* plants was measured under natural $T_{air}$ and $CO_2$ concentrations with an open gas-exchange system (*Li-6400*, *Li-Cor*, Lincoln, USA) that was operated at a flow rate of 500 μmol $s^{-1}$. The instrument automatically records photosynthetic and main environmental factor parameters. We carried out the measurements once in every hour from 8:00 to 20:00 h in accordance with the procedure proposed by Tomlinson (1990).

Gas-exchange parameters were measured in three fully expanded well-grown functional leaves from three different plants (*n* = 3) under each light regime, and each measurement were repeated thrice.

Table 1. Mean sizes and detailed habitats of 20 adult *S. hexandrum* plants. Each value denotes the mean (± SD) of samples [*n* = 8, 7, 5 for low (LL), medium (ML), and high light (HL), respectively] under the same light regime.

| Light regime | No. of plants | Height [cm] | Crown diameter [cm] | Leaf number per plant | Detailed habitats |
|---|---|---|---|---|---|
| LL | 8 | 56.32 ± 8.32 | 47.56 ± 5.32 | 2.67 ± 0.79 | Close to shrubs under the shade throughout the day |
| ML | 7 | 67.21 ± 9.16 | 65.42 ± 4.87 | 3.13 ± 1.02 | Nearby shrubs under the shade for about half a day |
| HL | 5 | 53.75 ± 8.42 | | | Open places with no shade |

**Environmental data and statistical analyses**: During the diurnal course, all the relative environmental data were recorded in each step by using *Li-6400*, including vapour pressure deficit of air (VPD$_{air}$). The ecological suitability of *S. hexandrum* was assessed using one-way analysis of variance (*ANOVA*). Means were compared using the *Duncan*'s test under three light regimes. *Pearson*'s correlation was used to assess the relationship between parameters. Statistical analyses and figure plotting were performed using *SPSS 18.0* (*SPSS Inc.*, Chicago, USA) and *Sigmaplot 11.0* (*Systat Software, Inc.*), respectively.

## Results

**Morpho-anatomical leaf traits**: LA and LP showed the same change trend under three light regimes, which were ranked in the following order: moderate light (ML) > low light (LL) > high light (HL) (Table 2). SLM, LT, and Chl also showed higher values for ML than those for HL and LL, which also showed significant differences. Stomata were observed only on the lower leaf surface (hypostomatous leaves). The values of S$_d$ for LL were 21.5 and 24.7% higher than those in ML and HL, respectively. The smallest S$_s$ value was observed under LL (Table 2).

**P$_N$-PAR response curves and fitting parameters**: Significant difference was observed between the photosynthetic parameters of *S. hexandrum* under the three conditions. P$_N$ was higher under LL and HL than that under ML with the lower PAR [from 0 μmol m$^{-2}$ s$^{-1}$ to approximately 240 μmol(photon) m$^{-2}$ s$^{-1}$] (Fig. 1). However, with the increase in PAR [from 240 to 1,600 μmol(photon) m$^{-2}$ s$^{-1}$], P$_N$ under different light conditions was ranked in the following order: ML > HL > LL (Fig. 2). The fitting parameters, such as P$_{Nmax}$, α, L$_{CP}$, L$_{SP}$, and R$_D$, reached the maximum values under ML (Table 3). The α

under LL and ML, P$_{Nmax}$ under HL and LL, and L$_{SP}$ under HL and ML showed no significant differences (Table 3).

**Diurnal variation of photosynthesis parameters under natural conditions**: Fluctuations in the main environmental factors (Fig. 3) and the photosynthetic parameter variation throughout the course of the day (Fig. 4) were measured. The highest T$_{air}$ of approximately 23.4°C was observed at 14:00 h (12 July) (Fig. 3), with the highest VPD$_{air}$ values (1.45 kPa) (Fig. 3). The most intense illuminations of 658; 1,348; and 1,855 μmol(photon) m$^{-2}$ s$^{-1}$ were also recorded at 14:00 h for LL, ML, and HL, respectively (Fig. 3).

Diurnal variations in P$_N$ were single-peak curves with minor fluctuations under the three light regimes. P$_N$ under ML was higher than those under LL and HL, except that at 8:00–9:00 h. P$_N$ under HL was higher at 8:00–11:30 and 17:30–20:00 h compared to that of LL. P$_N$ peaks appeared at nearly 16:00, 15:00, and 11:00 h under LL, ML, and HL conditions, respectively (Fig. 4A). The g$_s$ values under LL and ML were also higher than that in HL. In particular, the g$_s$ peaks for LL and HL appeared at around 14:00 h and that for ML occurred at 11:00 h (Fig. 4B). The transpiration rate (E) for LL was lower than those of the others during day time (Fig. 4C). During the diurnal variation, the values of WUE$_i$ for ML and HL were higher than that of LL. They kept increasing with some fluctuations from 8:00 to 16:00 h in three light regimes, and then reached the peak values. Therefore, they decreased from 16:00 to 20:00 h (Fig. 4D).

The correlation results between P$_N$ and WUE$_i$, Chl, S$_s$, PAR, T$_{air}$, and VPD$_{air}$ in *S. hexandrum* were significant (Table 4). Nonetheless, no correlation was observed between P$_N$ and g$_s$, SLM, or S$_d$. A correlation was also detected between WUE$_i$ and g$_s$, SLM, S$_d$, S$_s$, and PAR, and between SLM and Chl, S$_d$, S$_s$, and PAR (Table 4). Associations were also observed between S$_d$ and Chl, S$_s$, g$_s$, and PAR and between Chl and S$_s$ (Table 4). Among the

Table 2. Leaf area and perimeter (LA and LP), specific leaf mass (SLM), leaf thickness (LT), chlorophyll content (Chl), stomatal density and size (S$_d$ and S$_s$) of nine *S. hexandrum* plants under three light regimes. Each value denotes the mean (± SD) of three plants (*n* = 3) under the same regimes. Means within columns followed by *different small letters* are statistically significant at the 0.05 level.

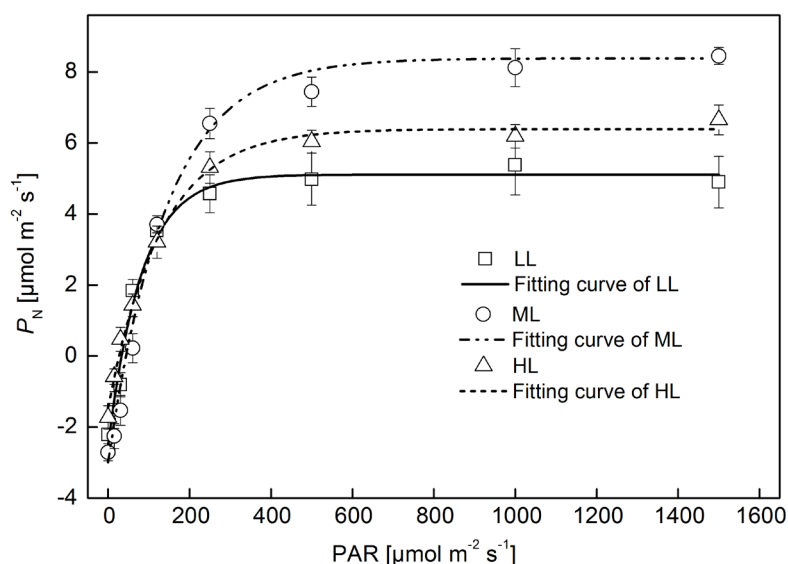| Light regime | LA [cm$^2$] | LP [mm] | SLM [mg cm$^{-2}$] | LT [mm] | Chl [mg g$^{-1}$] | S$_d$ [mm$^{-2}$] | S$_s$ [μm] |
|---|---|---|---|---|---|---|---|
| LL | 311.62 ± 12.43[b] | 151.46 ± 8.43[b] | 3.03 ± 0.43[c] | 0.48 ± 0.15[c] | 3.09 ± 0.41[c] | 82.67 ± 6.43[a] | 43.79 ± 10.42[b] |
| ML | 411.87 ± 23.01[a] | 201.89 ± 10.25[a] | 5.61 ± 0.31[a] | 0.85 ± 0.11[a] | 5.27 ± 0.45[a] | 64.89 ± 8.23[b] | 51.93 ± 9.42[a] |
| HL | 198.46 ± 21.42[c] | 124.92 ± 9.36[c] | 4.47 ± 0.28[b] | 0.67 ± 0.13[b] | 3.69 ± 0.50[b] | 62.22 ± 7.85[b] | 51.20 ± 11.03[a] |
| *p* | 0.001 | 0.003 | 0.000 | 0.000 | 0.001 | 0.001 | 0.000 |

Fig. 1. Response curves of photosynthesis ($P_N$)-photosynthetically active radiation (PAR) of *S. hexandrum* under low light (LL), medium light (ML), and high light (HL) regimes. Each value denotes the mean ($\pm$ SD) of three plants ($n = 9$) in the same light regimes.



Regression equations:
LL : y = 0.039 x -1.69 $R^2$ = 0.892 $n$ = 3
ML: y = 0.040 x -2.42 $R^2$ = 0.953 $n$ = 3
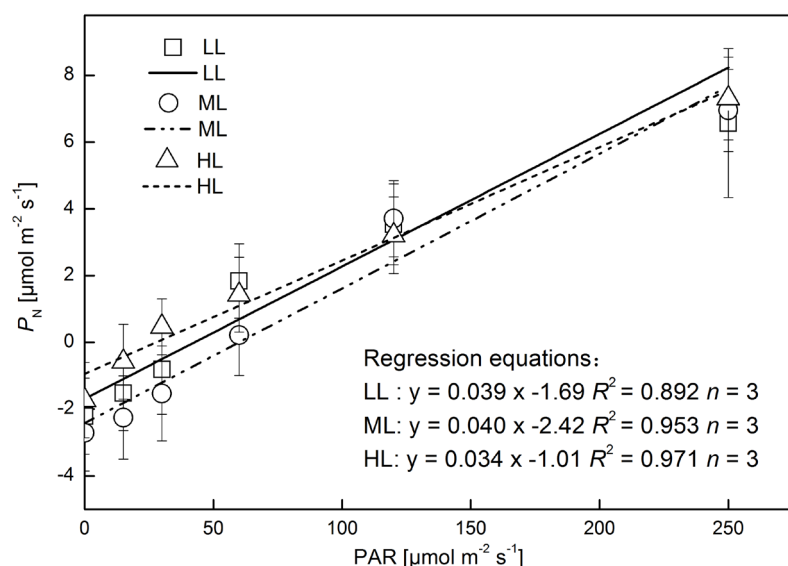HL: y = 0.034 x -1.01 $R^2$ = 0.971 $n$ = 3

Fig. 2. Regression equations of photosynthesis ($P_N$) – photosynthetically active radiation (PAR) of *S. hexandrum* under weak light level under low light (LL), medium light (ML), and high light (HL) regimes. Each value denotes the mean ($\pm$ SD) of three plants ($n = 9$) in the same light regimes.

Table 3. Fitting values of net photosynthetic rate ($P_N$)-photosynthetically active radiation (PAR) curves of *S. hexandrum* plants under three light regimes. Values ($\pm$ SD) ($n = 9$) within columns followed by *different small letters* are statistically significant at the 0.05 level.

| Light regime | $P_{Nmax}$ [µmol m$^{-2}$ s$^{-1}$] | $\alpha$ | $L_{SP}$ [µmol m$^{-2}$ s$^{-1}$] | $L_{CP}$ [µmol m$^{-2}$ s$^{-1}$] | $R_D$ [µmol m$^{-2}$ s$^{-1}$] |
|---|---|---|---|---|---|
| LL | 5.36 ± 0.23[b] | 0.039 ± 0.005[a] | 868.23 ± 26.31[b] | 34.51 ± 5.43[b] | 1.69 ± 0.78[b] |
| ML | 9.31 ± 0.57[a] | 0.040 ± 0.003[a] | 1030.26 ± 28.04[a] | 54.87 ± 5.92[a] | 2.42 ± 0.91[a] |
| HL | 6.38± 0.61[b] | 0.034 ± 0.004[b] | 989.87 ± 19.52[a] | 15.09 ± 2.16[c] | 1.01 ± 0.28[c] |
| *p* | 0.000 | 0.001 | 0.000 | 0.003 | 0.002 |

environmental factors, the relationships of PAR, $T_{air}$, and $VPD_{air}$ were significantly correlated with one another. In particular, a significant correlation was observed between PAR and $S_s$ (Table 4). Positive correlations were also observed between $P_N$ and $WUE_i$, Chl, $S_s$, PAR, $T_{air}$ or $VPD_{air}$; between $g_s$ and $S_d$; between $WUE_i$ and SLM, $S_d$ or PAR; and between $S_s$ and SLM, Chl or PAR. SLM was correlated with Chl and PAR. On the contrary, a negative correlation was detected between $WUE_i$ and $S_s$. In addition, $S_d$ was associated with Chl, $S_s$ and PAR.

## Discussion

Leaf traits showed significant differences with increased/

decreased illumination (Petter *et al.* 2016, Yannelli *et al.* 2017). For some plants, HL would result in small LA and large LT (Terashima *et al.* 2001, Pons 2016), and thus large SLM (Puglielli *et al.* 2017). On the contrary, the thin and large leaves were usually produced under LL habitats (Portsmuth and Niinemets 2007). In the meantime, Chl was also sensitive to the light conditions (Sato *et al.* 2015).

For example, most plants could not synthesize Chl due to the lack of the progenitor cells without sufficient light (Duanmu *et al.* 2013). However, HL could induce plant leaves to reduce Chl synthesis (Li *et al.* 2014), which could be a self-protection mechanism to avoid absorbing too much light energy and photoinhibition (Tucci *et al.* 2010). By contrast, certain shade (ML) was beneficial to Chl
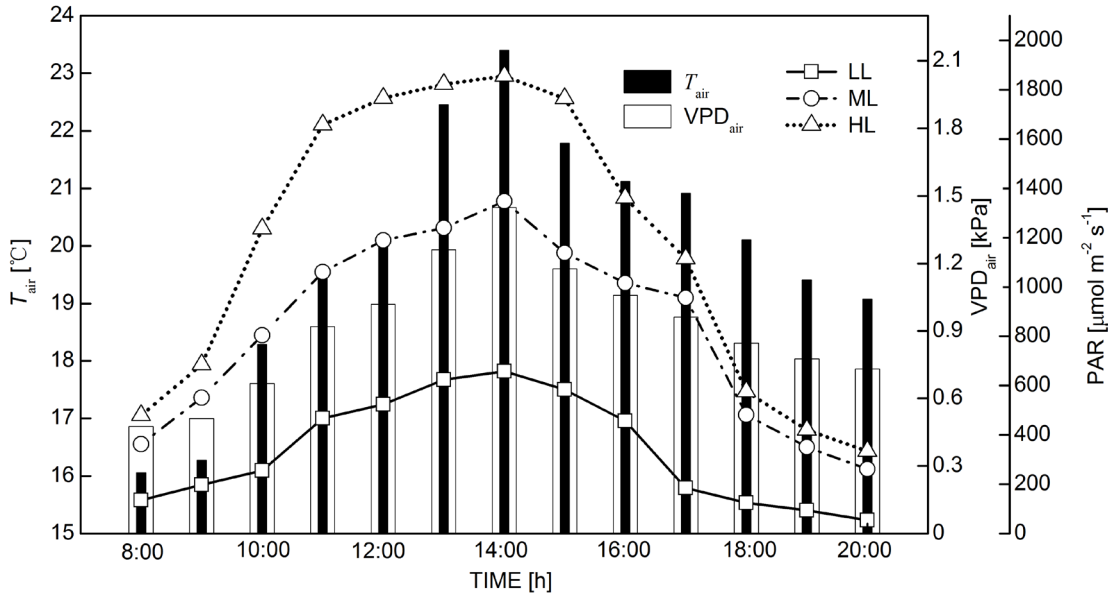


Fig. 3. Diurnal variation in air temperature ($T_{air}$), vapour pressure deficit of air ($VPD_{air}$), and photosynthetically active radiation (PAR) under low light (LL), medium light (ML), and high light (HL) regimes on 12 July 2015 (Butterfly Valley, Bujiu Town, Nyingchi County, Southeast Tibet of China).
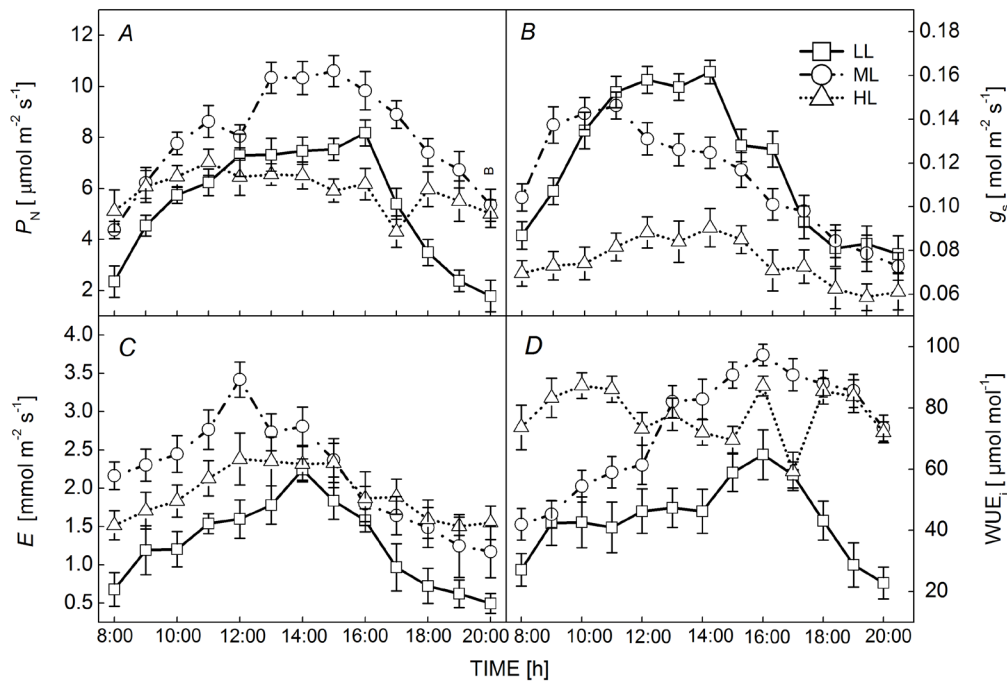


Fig. 4. Diurnal variation in net photosynthetic rate ($P_N$) (*A*), stomatal conductance ($g_s$) (*B*), transpiration rate (*E*) (*C*), and intrinsic water-use efficiency ($WUE_i$) (*D*) of *S. hexandrum* under low light (LL), medium light (ML), and high light (HL) regimes. Each value denotes the mean (± SD) of three plants (*n* = 9) in the same light regimes.

Table 4. *Pearson*'s correlation coefficients (upper right section) and *p* values (lower left section) in terms of the leaf traits, photosynthetic parameters, and the main environmental factors. Significant differences at the 0.05 level (*n* = 117) are marked *in bold*.

| | $P_N$ | $g_s$ | $WUE_i$ | SLM | Chl | $S_d$ | $S_s$ | PAR | $T_{air}$ | $VPD_{air}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| $P_N$ | - | 0.244 | **0.538** | 0.231 | 0.519 | −0.020 | 0.377 | 0.572 | 0.508 | 0.563 |
| $g_s$ | 0.135 | - | **−0.614** | −0.242 | 0.238 | 0.332 | −0.296 | −0.098 | 0.079 | 0.179 |
| $WUE_i$ | 0.000 | 0.000 | - | 0.583 | 0.259 | **−0.399** | 0.580 | 0.530 | 0.307 | 0.264 |
| SLM | 0.206 | 0.137 | 0.000 | - | 0.710 | **−0.660** | 0.820 | 0.591 | 0.073 | 0.094 |
| Chl | 0.001 | 0.144 | 0.111 | 0.000 | - | **−0.378** | 0.580 | 0.283 | 0.025 | 0.071 |
| $S_d$ | 0.905 | 0.039 | 0.012 | 0.000 | 0.018 | - | **−0.635** | **−0.345** | 0.187 | 0.155 |
| $S_s$ | 0.018 | 0.068 | 0.000 | 0.000 | 0.000 | 0.000 | - | 0.540 | 0.011 | 0.045 |
| PAR | 0.000 | 0.555 | 0.001 | 0.000 | 0.081 | 0.031 | 0.000 | - | 0.498 | 0.581 |
| $T_{air}$ | 0.001 | 0.634 | 0.057 | 0.657 | 0.881 | 0.254 | 0.945 | 0.001 | - | 0.973 |
| $VPD_{air}$ | 0.000 | 0.276 | 0.105 | 0.570 | 0.666 | 0.345 | 0.788 | 0.000 | 0.000 | - |

synthesis and area expansion; thus, the leaves with a high Chl content and SLM had large LA. Similar results had been reported in *Campanulastrum americanum* (Galloway *et al*. 2009), *Syringa oblata* (Xiao *et al*. 2015), and *Olea europaea* (Gregoriou *et al*. 2007). Our findings were consistent with these results. Therefore, the preference of *S. hexandrum* was certain shade conditions.

Stomatal characteristics were sensitive to heterogeneous light regimes in several herb species (Magyar *et al*. 2007). In other plant species (*e.g. Taraxacum mongolicum, Arabidopsis thaliana*, and *S. oblata*), an increase in PAR induced an increase in $S_d$ and a decrease in $S_s$ (Pigliucci *et al*. 2002, Zhao *et al*. 2007, Xiao *et al*. 2015). However, $S_d$ and $S_s$ of *S. hexandrum* leaves were reduced and increased under LL and HL habitats, respectively (Table 2). A significant negative/positive correlation was observed between PAR and $S_d$/$S_s$ (Table 4). Shaded samples displayed developed mechanisms, which ultimately aimed to maximise carbon gain through their ontogenetic stages towards the canopy (Kenzo *et al*. 2012). The strategies involved the increased stomatal density ($S_d$) and nutrient assimilation and decreased $S_s$; small stomata responded to extreme environment that drives stomatal movements to avoid water loss (Meinzer and Grantz 1990). Our data supported the hypothesis that $S_d$ was significantly positively/ negatively correlated with $g_s$ ($r$ = 0.332, $p<0.05$)/$WUE_i$ ($r = −0.399, p<0.05$) and significantly negatively correlated with $S_s$ ($r = −0.635, p<0.05$). In particular, the maximum $S_d$ and $S_s$ also appeared under ML. These results indicated that a certain shading conditions promoted the stomatal development of *S. hexandrum*. These findings were similar to those in shade-tolerant plant *Trifolium repens* (Huber *et al*. 2008*)*. The similarity might be caused by the adaptation of phenotypic traits and acclimatisation (Pilahome *et al*. 2017*)*.

The results of the $P_N$-PAR response curves, showed that the high α and $R_D$ of LL and ML suggested that certain shade promoted the light energy transformation and nutrient consumption in plant leaf, which were contrary to those under HL. Overall, lowered $L_{CP}$ [≤55 μmol(photon) m$^{-2}$ s$^{-1}$] illustrated that *S. hexandrum* possessed a certain shade tolerance and the presence of high $L_{SP}$ [approximately 1,000 μmol(photon) m$^{-2}$ s$^{-1}$] in

herbs (Albrecht and McCarthy 2009).

During diurnal variations, $P_N$ and $E$ under ML maintained higher values than those under LL and HL (Figs. 4*A,C*), with $g_s$ reduced from 11:00 to 20:00 h. These results demonstrated that ML was suitable for carbon assimilation and solar energy utilization (Mozzo *et al*. 2008). Furthermore, much water evaporation from leaf epidermis increased the heat dissipation to maintain the stability of blade temperature (Long *et al*. 1994, Müller *et al*. 2001, Baker 2008). Plant reduced $g_s$ and $E$ to avoid water loss during the high irradiance period (La Rocca *et al*. 2015), whilst the activated photosynthetic enzymes could generate the increasing of $P_N$ quickly (Zhang *et al*. 2000). Therefore, the change trends of $P_N$ and $g_s$ were nearly synchronous under HL during the diurnal variations (Figs. 4*A,B*). Simultaneously, the special shade-tolerant property of *S. hexandrum* could keep the high ability of carbon assimilation in mesophyll cell under ML and LL (Fini *et al*. 2010), but $g_s$ decreased during the midday period (Figs. 4*A,B*). Therefore, the main determinant of *S. hexandrum* photosynthesis should be a nonstomatal factor (Jifon *et al*. 2003). In general, $g_s$ was not the main regulator for $P_N$ because $g_s$ and $P_N$ were insignificantly correlated (r = 0.244, $p>0.5$). Shade in LL and ML significantly delayed the times of $P_N$ peak emergence compared to that under HL, which significantly promoted the increase in $g_s$ (Fig. 4*B*). These results could occur due to the high $S_d$ under shaded conditions (Table 2). Although LL or HL was unfavourable for plant growth, due to low $E$ and $WUE_i$, such light conditions could enable the plants to adopt the corresponding strategies for survival (Valladares and Niinemets 2008).

In general, the main environmental factors significantly influenced SLM, $S_s$, and the photosynthetic characteristics of *S. hexandrum*. Several significant correlations were also observed between PAR and $P_N$, SLM and $S_s$, which indicated that a certain PAR value was beneficial for photosynthesis, mesophyll cell (SLM), and stomatal development (Sawhney and Zelitch 1969, Lawson *et al*. 2014). $T_{air}$ was significantly positively correlated with $P_N$, which might be due to the cold plateau climate (Davison 1991). Moreover, a change in $T_{air}$ from 16.0 to 23.4°C was observed during diurnal variation

(Fig. 2). VPD$_{air}$ positively affected $P_N$. According to atmospheric properties, high VPD$_{air}$ presented the relatively dry air, which enhanced the gas-exchange capacity through the stomata. The same results were reported by Rawson et al. (1977) and Suzuki et al. (2015).

In conclusion, our results showed that the ML regime was the best habitat for the endangered S. hexandrum based on the leaf traits and photosynthetic performance, such as the highest values of LA, SLM, Chl, and S$_s$, and the most active metabolism, such as $P_{Nmax}$, $E$, and $R_D$. Therefore, appropriate measures should be taken to promote the propagation and recovery of wild populations in the future. Firstly, the suitable habitats of S. hexandrum populations, such as shrubbery, and large-scale gaps, are the key protection areas, and the approach of in situ conservation is feasible. Some measures, including excessive overexploitation and collecting seeds, should be strictly forbidden. Secondly, some habitats with poor light transmittance can be partly cleared of trees and shrubs to improve light regimes and atmospheric conditions. Thirdly, the cultivated S. hexandrum should be supported energetically and popularised to supply/meet the market demand.

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