

# Temperature acclimation of photosynthesis in *Meconopsis horridula* var. *racemosa* Prain.

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**ABSTRACT.** *Meconopsis horridula* var. *racemosa* Prain. is a famous alpine flower and medicinal plant native to high elevations in the Himalayas, but cultivating it at lower altitudes presents great challenges. The photosynthetic gas exchange and chlorophyll fluorescence of *M. horridula* were investigated at three temperatures to evaluate its photosynthetic performance and the relative importance of biochemical limitation, stomatal limitation, and mesophyll limitation to photosynthesis under different temperature regimes. *Meconopsis horridula* grown at 20°C could obtain the highest photosynthetic rate and photochemical efficiency among the three temperatures, and photosynthetic performance at low temperature was better than at high temperature. Non-photochemical quenching was an important mechanism protecting the photosynthetic apparatus of *M. horridula* under temperature stress conditions. Although mesophyll conductance was the dominant factor for limiting photosynthesis of *M. horridula* both at low temperature and high temperature, the photosynthesis at high temperature was also limited by stomatal conductance and biochemical efficiency. The poor photosynthetic performance at high temperature may be what limits *M. horridula* cultivation at low altitude.

**Keywords:** Chlorophyll fluorescence; High temperature; *Meconopsis horridula*; Photosynthesis; Photosynthetic limitation.

## INTRODUCTION

The majority of the 49 species in the genus *Meconopsis* belonging to Papaveraceae grow at high elevations (2,100-5,780 m) in the Himalayas and other mountains in western China. Only *M. cambrica* can be found in Europe (Chuang, 1981). As famous horticultural plants bearing beautiful flowers, *Meconopsis* has attracted the attention of botanists. Some *Meconopsis* species are also used as traditional herbal medicine for their anti-inflammatory and analgesic activities (Samant et al., 2005). The gene resources of *Meconopsis* have been increasingly threatened due to habitat destruction and over-harvesting of the plants from the natural habitats (Sulaiman and Babu, 1996). Several members of *Meconopsis* have been cultivated for over 100 years, but cultivating *Meconopsis* is not an easy task (Still et al., 2003).

Empirical observation suggests that high temperature during the growing season is an important determinant limiting the growth and development of *Meconopsis* (Norton and Qu, 1987; Ren, 1993). Both *M. punicea* and *M. betonicifolia* grown at 7.2°C /10°C (night/day temperature) have larger dry weight and flower size than at 18.3°C /21.1°C (Still et al., 2003). *Meconopsis integrifolia* can flower in its native habitat with snow on the ground. This

remarkable tolerance to low temperature may result in the poor adaptability to high temperature. The growth and survival of plants can be limited by the thermo-tolerance capabilities of photosynthesis as photosynthetic traits govern carbon acquisition (Sharkey, 2000).

Several mechanisms for thermal acclimation of photosynthesis have been proposed. Plants grown at low temperatures have higher levels of Rubisco and other enzymes, which are involved in carbon metabolism compared with plants grown at high temperatures. Growth at low temperatures also results in higher levels of cytosolic fructose-1,6-bisphosphatase and sucrose-phosphate synthase, which regenerates orthophosphate during sucrose synthesis (Strand et al., 1999; Hikosaka et al., 2006). On other hand, high temperatures have been shown to alter thylakoid membrane structure, decrease Rubisco activity and RuBP regeneration capacity, perturb photosynthetic electron transport, increase dark respiration and photorespiration, and sequentially affect carbon assimilation (Yamasaki et al., 2002; Streb et al., 2003; Wise et al., 2004). However, the photosynthetic protective mechanisms vary greatly between plant species and ecotypes (Yamasaki et al., 2002). Unfortunately, little is known about the photosynthetic adaptation of *Meconopsis* to temperature. Such knowledge is particularly relevant to the domestication of wild species, the purpose of which is to protect wild populations from over-harvesting.

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The growth and development of plants depend on their physiological suitability to the growth environment they inhabit (Wu and Campbell, 2006). Chlorophyll fluorescence and photosynthetic measurements are widely used in predicting plant performance and physiological tolerances to the environment (Hamerlynck and Knapp, 1996; Zhang et al., 2006) and can be employed to study the physiological adaptations of alpine plants to changing temperatures.

*Meconopsis horridula* var. *racemosa* (Maxim.) Prain is a perennial herb with sharp spines on its leaves and stems. It occurs on rocky slopes at altitudes between 3,000 and 4,900 m in southwestern China. This species blooms from June to July, and bears fruit from July to September. In the present study, the photosynthetic gas exchange and chlorophyll fluorescence of *M. horridula* were investigated under different temperatures. The main goal was to evaluate its photosynthetic performance and to elucidate the relative importance of three major limitations to photosynthesis in *M. horridula* under different temperature regimes.

## MATERIALS AND METHODS

### Plant materials and temperature treatments

The seeds of *M. horridula* var. *racemosa* were collected from Hong Mountain in southwestern China at an altitude of 3,900 m and were sown and cultivated in a nursery. In March 2007, one-year-old dormant plants were grown in plastic pots with sand, loam, and humus (2:2:1, v/v/v) under ambient conditions. After the seedlings emerged, thirty seedlings were transferred to growth chambers, where the temperatures were maintained at 30°C, 20°C and 10°C, respectively. In the growth chambers, the photosynthetic photon flux density (PPFD) was 500  $\mu\text{mol m}^{-2}\text{s}^{-1}$  with a photoperiod of 12 h, and the average relative humidity was about 62%. The seedlings were fertilized with a liquid nutrient solution at 15-day intervals and watered every 5-7 days as needed. The measurements were performed June 3-16, 2007.

### Measurement of photosynthesis

Photosynthesis and chlorophyll fluorescence in response to PPFD and  $\text{CO}_2$  concentration was measured on the fully expanded leaves using a Li-6400 portable photosynthesis system with a chlorophyll fluorescence chamber (Li-Cor Ltd., Lincoln, NE, USA). Before measurement, the leaf was adapted in darkness more than 10 h. Dark respiration ( $R_{\text{dark}}$ ) was measured at ambient  $\text{CO}_2$  concentration of 370  $\mu\text{mol mol}^{-1}$ . After the minimum fluorescence ( $F_0$ ) was determined by a weak modulated light, a 0.8 s saturating light was used on dark-adapted leaf to determine the maximum fluorescence ( $F_m$ ). The leaf was then illuminated by an actinic light of 1200  $\mu\text{mol m}^{-2}\text{s}^{-1}$  (10% blue, 90% red) for 15 min. The response curves of photosynthetic rate ( $P_n$ ) and chlorophyll fluorescence to PPFD were made using an automated protocol built into Li-6400. Each leaf was equilibrated to initial conditions by waiting at least 15 min before executing the automated protocol.  $P_n$ -PPFD curves

of five leaves were measured at 13 light intensities under controlled levels of  $\text{CO}_2$  (370  $\mu\text{mol mol}^{-1}$ ), flow rate (500  $\text{mmol s}^{-1}$ ) and vapor pressure deficit (1.0-1.5 kPa). Leaf temperatures were adjusted respectively to 10, 20 and 30°C depending on the growth temperature.

Photosynthetic  $\text{CO}_2$  response curves ( $P_n$ - $C_i$ ) and  $P_n$ -PPFD curves were determined on the same leaves. After completion of a  $P_n$ -PPFD curves measurement, the leaf was induced at 1200  $\mu\text{mol m}^{-2}\text{s}^{-1}$  PPFD and 370  $\mu\text{mol mol}^{-1}$   $\text{CO}_2$  concentration for 15 min. Photosynthetic rates versus  $\text{CO}_2$  response curves together with chlorophyll fluorescence were measured at PPFD of 1200  $\mu\text{mol m}^{-2}\text{s}^{-1}$ . The settings of leaf temperature and vapor pressure deficit were the same as those of the  $P_n$ -PPFD curve measurement. The measurements of  $P_n$ - $C_i$  curves were started at an ambient  $\text{CO}_2$  concentration, which was decreased gradually to 0  $\mu\text{mol mol}^{-1}$  and then increased to ensure that the stomata stayed open throughout the measurement. The photosynthetic rate and chlorophyll fluorescence were measured at different  $\text{CO}_2$  concentrations using the automated protocol built into the Li-6400.

### Calculations of parameters

The chlorophyll fluorescence parameters were calculated as: (1) potential quantum yield of PSII:  $F_v/F_m = (F_m - F_0)/F_m$ ; (2) effective quantum yield of PSII:  $\phi_{\text{PSII}} = (F_m' - F_s)/F_m'$ , where  $F_s$  is steady-state fluorescence and  $F_m'$  is maximum fluorescence in the light; (3) efficiency of excitation energy capture by open reaction centre:  $F_v'/F_m' = (F_m' - F_0')/F_m'$ ,  $F_0'$  is minimum fluorescence in the light; (4) apparent rate of electron transport of PSII:  $J_{\text{ETR}} = 0.5\phi_{\text{PSII}}Q_{\text{abs}}$ , where  $Q_{\text{abs}}$  was the absorbed light energy that was calculated as PPFD\*leaf absorbance, and leaf absorbance was taken as 0.85; (5) photochemical quenching:  $qP = (F_m' - F_s)/(F_m' - F_0')$ ; (6) non-photochemical quenching:  $NPQ = (F_m - F_m')/F_m'$ .

$P_n$ -PPFD curves were fit by a non-rectangular hyperbola. Light saturated photosynthetic rate ( $P_{\text{max}}$ ), day respiration ( $R_{\text{day}}$ ) and apparent quantum yield (AQE) were determined for each leaf using Photosyn Assistant v.1.1 (Dundee Scientific, Scotland, UK), which follows the estimation method of Prioul and Chartier (1977).

The mesophyll conductance ( $g_m$ ) was estimated according to the method of Harley et al. (1992a) as

$$g_m = \frac{P_n}{C_i - \frac{\Gamma^* [J_{\text{ETR}} + 8(P_n + R_{\text{day}})]}{J_{\text{ETR}} - 4(P_n + R_{\text{day}})}}$$

where  $R_{\text{day}}$ , the rate of day respiration, was calculated from the  $P_n$ -PPFD curve on the same leaf. The  $\text{CO}_2$  compensation points in the absence of respiration ( $\Gamma^*$ ) at given temperatures were derived from the value at 25°C (42.75  $\mu\text{mol mol}^{-1}$  in tobacco) according to the method of Bernacchi et al. (2001). Mesophyll conductance was calculated from the  $P_n$  at  $C_i$  100-300  $\mu\text{mol mol}^{-1}$ , and the average value of  $g_m$  was determined for each leaf (Niinemets et al., 2005).

The rate of photosynthetic electron transport ( $J_{ETR}$ ) was obtained from chlorophyll fluorescence on the same leaf.

The  $CO_2$  concentration at carboxylation site,  $C_c$ , was calculated as

$$C_c = C_i - P_n / g_m$$

The biochemical capacity for photosynthesis can be examined using the response curve of photosynthesis to internal  $CO_2$  concentration ( $C_i$ ) and chloroplastic  $CO_2$  concentration ( $C_c$ ). The maximum carboxylation rate by Rubisco ( $V_{cmax}$ ), light-saturated electron transport ( $J_{max}$ ) and triose phosphate utilization (TPU) both on the basis of  $C_i$  and  $C_c$  were calculated by Photosyn Assistant software based on the photosynthetic model of von Caemmerer and Farquhar (1981). The Michaelis-Menten constant for  $CO_2$  ( $K_c$ ,  $404.9 \mu mol mol^{-1}$  at  $25^\circ C$ ) and for  $O_2$  ( $K_o$ ,  $278.4 mmol mol^{-1}$  at  $25^\circ C$ ) and  $\Gamma^*$  were taken from Bernacchi et al. (2001). The values at given temperatures were calculated according to the method of Bernacchi et al. (2001).

The relative limitation to take into account  $g_m$  to partition photosynthetic limitation was proposed by Jones (1985) and modified by Grassi and Magnani (2005). In this method, a reference which has highest  $P_n$  as a standard should be assumed, the values at  $20^\circ C$  was used as the references. The relative limitation of stomatal conductance ( $S_L$ ), mesophyll conductance ( $S_M$ ) and biochemical characteristics ( $S_B$ ) to photosynthesis were calculated as below (Grassi and Magnani, 2005).

$$S_L = l_s \cdot \frac{g_s^{ref} - g_s}{g_s^{ref}}$$

$$S_M = l_m \cdot \frac{g_m^{ref} - g_m}{g_m^{ref}}$$

$$S_B = l_b \cdot \frac{V_{cmax}^{ref} - V_{cmax}}{V_{cmax}^{ref}}$$

$$l_s = \frac{g_{tot} / g_s \cdot \partial P_n / \partial C_c}{g_{tot} + \partial P_n / \partial C_c}$$

$$l_m = \frac{g_{tot} / g_m \cdot \partial P_n / \partial C_c}{g_{tot} + \partial P_n / \partial C_c}$$

$$l_b = \frac{g_{tot}}{g_{tot} + \partial P_n / \partial C_c}$$

$$\frac{\partial P_n}{\partial C_c} = \frac{(1 + O / K_o) \cdot K_c \cdot V_{cmax} \cdot (C_c - \Gamma^*)}{((1 + O / K_o) \cdot K_c + C_c) \cdot ((1 + O / K_o) \cdot K_c + C_c) \cdot C_c} + \frac{V_{cmax} \cdot C_c \cdot \Gamma^*}{((1 + O / K_o) \cdot K_c + C_c) \cdot C_c \cdot C_c}$$

where  $g_{tot}$  is total conductance to  $CO_2$  between the leaf surface and carboxylation sites ( $1/g_{tot} = 1/g_s + 1/g_m$ ).  $g_s^{ref}$ ,  $g_m^{ref}$  and  $V_{cmax}^{ref}$  is the reference value of  $g_s$ ,  $g_m$  and  $V_{cmax}$  at  $20^\circ C$ , respectively.  $O$  is atmospheric  $O_2$  concentration.

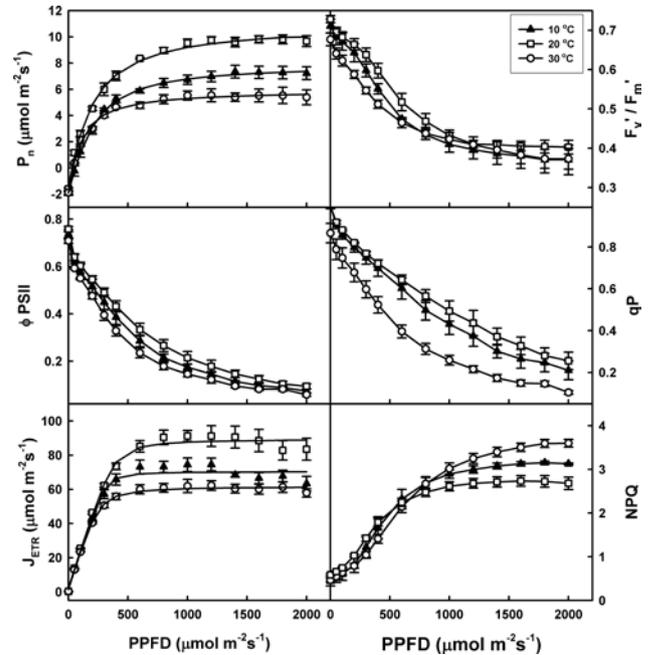
## Statistical analysis

Statistical analysis was performed using SPSS 12.0 for windows (SPSS Inc., Chicago, USA). The difference in photosynthetic variables among treatments was tested using one-way analysis of variance with an LSD test for post-hoc comparisons.

## RESULTS

Photosynthetic rate in *M. horridula* increased with PPFD at all temperatures (Figure 1). There were no significant differences in AQE and  $R_{dark}$  among temperatures, but the effect of growth temperature on  $P_{max}$  was pronounced and the  $R_{day}$  at  $10^\circ C$  was slightly higher than at  $30^\circ C$  (Table 1). The plants at  $20^\circ C$  had the highest  $P_{max}$  among treatments. Compared with the values of  $P_{max}$  in *M. horridula* at  $20^\circ C$ , the  $P_{max}$  was decreased by 19.6% at  $10^\circ C$  and by 36.4% at  $30^\circ C$ . At any temperatures, the values of  $F_v'/F_m'$ ,  $\phi PSII$  and  $qP$  decreased with increasing PPFD, while  $J_{ETR}$  and NPQ increased with PPFD. There were significant differences in  $qP$  ( $p < 0.01$ ) and  $J_{ETR}$  ( $p < 0.01$ ) among treatments. At low irradiance, NPQ was little changed, but above  $1000 \mu mol m^{-2} s^{-1}$  the difference increased among treatments (Figure 1).

The  $P_n$ - $C_i$  curves indicated that *M. horridula* grown at  $20^\circ C$  had a higher light-saturated rate of electron transport ( $J_{max}$ ) and triose phosphate utilization (TPU) both on the



**Figure 1.** Photosynthetic and chlorophyll fluorescence light response curves for *Meconopsis horridula* grown at different temperature regimes. Each point represents the mean  $\pm$  1 SE of five measurements.  $P_n$ , photosynthetic rate; PPFD, photosynthetic photon flux density;  $\phi PSII$ , effective quantum yield of PSII;  $F_v'/F_m'$ , efficiency of excitation energy capture by open reaction centre;  $J_{ETR}$ , apparent rate of electron transport of PSII;  $qP$ , photochemical quenching; NPQ, non-photochemical quenching.

**Table 1.** Photosynthetic parameters of *Meconopsis horridula* grown at three temperature regimes.

	Temperature regimes			<i>p</i>
	10°C	20°C	30°C	
$P_{\max}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	9.91±0.83ab	12.33±0.79a	7.84±0.69b	<0.05
$R_{\text{day}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	1.94±0.18a	1.36±0.12b	1.86±0.17ab	>0.05
$R_{\text{dark}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	2.94±0.46a	3.18±0.47a	3.66±0.42a	>0.05
AQE	0.038±0.006a	0.049±0.004a	0.056±0.006a	>0.05
$C_{i\text{-ambient}}$ ( $\mu\text{mol mol}^{-1}$ )	256.7±4.3a	243.0±8.5a	257.0±2.7a	>0.05
$C_{c\text{-ambient}}$ ( $\mu\text{mol mol}^{-1}$ )	161.7±11.8a	210.6±9.1b	193.3±6.7ab	<0.05
$V_{\text{cmax-ci}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	16.93±0.28a	30.60±2.25b	38.50±2.22c	<0.01
$V_{\text{cmax-cc}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	20.77±1.36a	34.90±3.52b	57.57±3.55c	<0.01
$J_{\text{max-ci}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	67.80±0.95a	105.90±9.27b	61.73±1.67a	<0.01
$J_{\text{max-cc}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	71.47±1.31a	110.77±8.16b	73.87±3.93a	<0.01
$J_{\text{max-ci}}/V_{\text{cmax-ci}}$	4.00±0.01a	3.45±0.08b	1.61±0.05c	<0.01
$J_{\text{max-cc}}/V_{\text{cmax-cc}}$	3.46±0.17a	3.09±0.09a	1.28±0.01b	<0.01
$J_{\text{ETR}}$ ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	69.03±4.18a	97.80±7.68b	61.07±6.88a	<0.05
TPU ( $\mu\text{mol m}^{-2}\text{s}^{-1}$ )	4.41±0.39a	6.99±0.71b	4.12±0.21a	<0.05

Different letters within the same row indicate mean values statistically different at  $p < 0.05$  as determined by LSD test.  $P_{\max}$ , light-saturated photosynthetic rate;  $R_{\text{day}}$ , respiration rate in the light;  $R_{\text{dark}}$ , respiration rate in dark; AQE, apparent quantum efficiency;  $C_{i\text{-ambient}}$ , intercellular  $\text{CO}_2$  concentration at ambient  $\text{CO}_2$  concentration;  $C_{c\text{-ambient}}$ , intercellular  $\text{CO}_2$  concentration at ambient  $\text{CO}_2$  concentration;  $V_{\text{cmax-ci}}$ , the  $C_i$ -based maximum RuBP saturated rate of carboxylation;  $V_{\text{cmax-cc}}$ , the  $C_c$ -based maximum RuBP saturated rate of carboxylation;  $J_{\text{max-ci}}$ , the  $C_i$ -based light saturated rate of electron transport;  $J_{\text{max-cc}}$ , the  $C_c$ -based light saturated rate of electron transport;  $J_{\text{ETR}}$ , apparent rate of electron transport of PSII; TPU, triose phosphate utilization.

basis of  $C_i$  and  $C_c$  for photosynthesis than those at 10°C and 30°C (Figure 2), while the maximum RuBP saturated rate of carboxylation ( $V_{\text{cmax}}$ ) increased with temperature. The values of  $J_{\text{max}}$  and  $V_{\text{cmax}}$  on the basis of  $C_i$  were lower than those on the basis of  $C_c$  indicating that the biochemical efficiencies were underestimated by using  $C_i$  (Table 1). The light-saturated electron transport rates ( $J_{\text{max}}$ ) calculated from  $P_n$ - $C_i$  curves were consistent with those ( $J_{\text{ETR}}$ ) derived from fluorescence-PPFD curves. Both on the basis  $C_i$  and  $C_c$ , the ratio of  $J_{\text{max}}$  to  $V_{\text{cmax}}$  decreased with the increasing temperature.

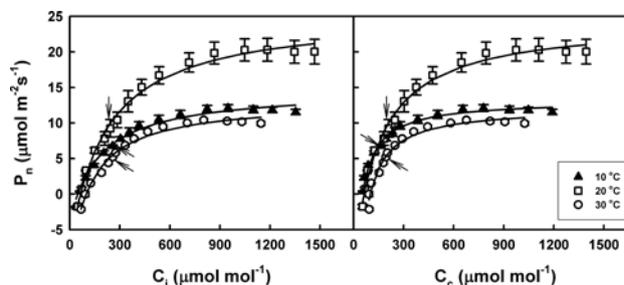
The values of  $F_v'/F_m'$ ,  $J_{\text{ETR}}$  and  $qP$  in *M. horridula* increased with PPFD on the whole (Figure 3), but the  $\text{CO}_2$  concentrations at which these values reached the maximal at 10°C and 20°C were higher than that at 30°C. The values of  $F_v'/F_m'$ ,  $J_{\text{ETR}}$  and  $qP$  at 10°C and 20°C were also higher than that at 30°C. The value of NPQ in *M. horridula* at 30°C was the highest, while the lowest at 20°C, suggesting that non-photochemical quenching was an important mechanism protecting the photosynthetic apparatus under temperature stress condition.

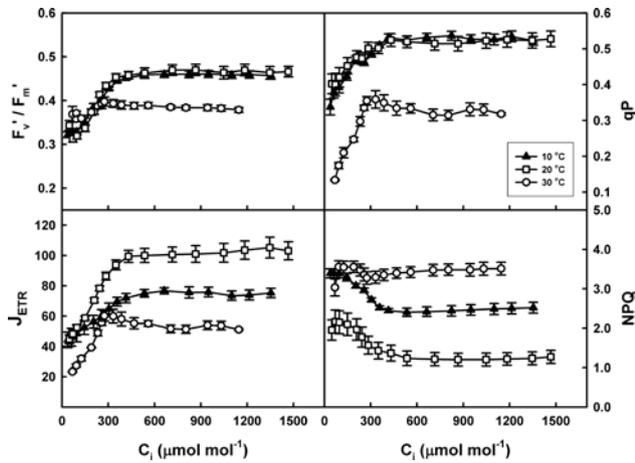
The values of  $g_s$  in *M. horridula* decreased with increasing temperature, but the plants at 20°C had higher  $g_m$  than those at 10°C and 30°C (Table 2). Quantitative limitation analysis showed that the photosynthetic limitation of *M. horridula* at 10°C came almost exclusively from mesophyll conductance while at 30°C it was the result of mesophyll conductance cooperating with biochemical and stomatal limitations (Table 2).

**Table 2.** Relative limitation analyses of *Meconopsis horridula* grown at three temperature regimes.

Treatment	$g_s$	$g_m$	Limitations (%)			
			$S_L$	$S_M$	$S_B$	Total
10°C	0.184±0.027	0.075±0.009	0	25.6	1.1	26.7
20°C	0.152±0.011	0.302±0.080	0	0	0	0
30°C	0.109±0.014	0.056±0.011	7.7	26.9	4.1	38.7

The values of plants grown at 20°C were used as the references.  $g_s$ , stomatal conductance;  $g_m$ , mesophyll conductance;  $S_L$ , stomatal limitation;  $S_M$ , mesophyll limitation;  $S_B$ , biochemical limitation.

**Figure 2.** Photosynthesis of *Meconopsis horridula* grown at different temperature regimes in response to intercellular  $\text{CO}_2$  concentration ( $C_i$ ) and chloroplastic  $\text{CO}_2$  concentration ( $C_c$ ). Each data point represents mean  $\pm$  1 SE of five measurements. The  $C_i$  and  $C_c$  at ambient  $\text{CO}_2$  concentration was marked by arrows.



**Figure 3.** Fluorescence parameters of *Meconopsis horridula* at different temperature regimes in response to intercellular  $\text{CO}_2$  concentration ( $C_i$ ). Each data point represents mean  $\pm$  1 SE of five measurements.  $F_v'/F_m'$ , efficiency of excitation energy capture by open reaction centre;  $J_{\text{ETR}}$ , apparent rate of electron transport of PSII;  $q_p$ , photochemical quenching; NPQ, non-photochemical quenching.

## DISCUSSION

Differences in photosynthetic adaptability are both genetically determined and environmentally induced (Hammerlynck and Knapp, 1996). Plants from habitats with different temperature regimes have different temperature response characteristics: the  $P_n$  at a temperature close to normal may be maximized, and plants grown in cold temperature regimes show maximum  $P_n$  at lower temperatures than do plants grown under warm temperatures (Berry and Björkman, 1980). *Meconopsis horridula* is native to the high mountains, which are characterized by low temperatures, and had a lower optimal photosynthetic temperature (Zhang and Hu, 2008). However, this remarkable tolerance to low temperature can lead to poor acclimation in warm temperatures. *M. horridula* showed the highest  $P_n$  at 20°C; meanwhile, the photosynthetic performance at low temperature was better than at high temperature.

The respiration rate ( $R_{\text{day}}$  and  $R_{\text{dark}}$ ) in *M. horridula* at ambient  $\text{CO}_2$  concentration was high. Alpine plants frequently have higher respiration rates than lowland plants (Atkin et al., 1996). Many studies have suggested that dark respiration increases with temperature (Law and Crafts-Brandner, 1999; Nogues et al., 2006). Respiratory losses are an important component of the carbon budget of alpine plants (Nogues et al., 2006). As a result, the high daytime respiration was partly responsible for the discrepancy between high  $V_{\text{cmax}}$  and low  $P_n$  at 30°C. However, the respiration rate in the light ( $R_{\text{day}}$ ) is lower than in darkness ( $R_{\text{dark}}$ ), indicating respiration was inhibited by light (Villar et al., 1994).

The photosynthetic performance of plants under stress conditions largely depends on their photosynthesis protection mechanisms. A major contributor to this protection is an increased ability to dissipate energy via non-photo-

chemical quenching, one of the mechanisms that protects chloroplasts from being damaged by excess excitation energy (Hjelm and Ögren, 2004). Stress conditions such as high temperature markedly promote non-photochemical quenching. At moderate light intensity, non-photochemical quenching is dependent on the temperature (Bilger and Björkman, 1991). The NPQ of *M. horridula* remained relatively high under high light and favorable temperature as well as under high temperature and suitable irradiance conditions, indicating that non-photochemical quenching was one of the important mechanisms protecting the photosynthetic apparatus under stress conditions.

Temperature influences photosynthesis primarily via enzyme activity as Rubisco activation is a primary site of inhibition (Feller et al., 1998; Law and Crafts-Brandner, 1999). Most plants have enzyme systems and membrane structures that are well matched to the temperature ranges they experience. As temperature increases, enzyme activity goes up. However, heat stress can decrease enzyme activity, and even cause the denaturation of proteins (Law and Crafts-Brandner, 1999). Low temperatures influence enzyme activity and the fluidity of the chloroplast membrane. As  $V_{\text{cmax}}$  is primarily regulated by Rubisco activity, it can reflect the activation state of Rubisco. In the present study, the maximum RuBP-saturated rate of carboxylation in *M. horridula* increased with temperature, indicating that the Rubisco activity of plants at 30°C was not inhibited significantly.

Photosystem II is quite sensitive to heat stress. Tang et al. (2007) suggested that heat stress can induce an aggregation of the light-harvesting complex of photosystem II, while Wise et al. (2004) suggested that photosynthetic electron transport is the functional limitation of photosynthesis at high temperature. In *M. horridula*, the  $J_{\text{ETR}}$  at 20°C was significantly higher than at 30°C, indicating that high temperature may inhibit the photosynthetic electron transport and the rate of electron transport was largely responsible for the photosynthetic performance of *M. horridula* at tested temperatures.

Based on the photosynthetic model of Farquhar et al. (1980), leaf photosynthesis is co-limited by RuBP carboxylation and RuBP regeneration. As the temperature dependence of RuBP carboxylation is different from that of RuBP regeneration, the balance between RuBP carboxylation and RuBP regeneration varies with growth temperature (Medlyn et al., 2002). However, the response of the  $J_{\text{max}}$  to  $V_{\text{cmax}}$  ratio to temperature was different among species. The  $J_{\text{max}}$  to  $V_{\text{cmax}}$  ratio in *Polygonum cuspidatum* increases with decreasing growth temperature (Onoda et al., 2005). The balance between carboxylation and regeneration of RuBP potentially affects the temperature dependence of photosynthesis (Medlyn et al., 2002). The ratio of  $J_{\text{max}}$  to  $V_{\text{cmax}}$  in *M. horridula* decreased with the increasing temperature, indicating that when the plants are grown at high temperature, the photosynthetic rate was limited by RuBP regeneration, while it was limited by RuBP carboxylation at low temperature.

The electron transport rate ( $J_{ETR}$ ) of *M. horridula* increased with increasing  $C_i$ , but the  $J_{ETR}$  of plants at 30°C above saturated  $C_i$  decreased slightly, indicating a triose phosphate utilization (TPU) limitation in *M. horridula* at 30°C (Table 1). Harley et al. (1992b) showed that the TPU-limited photosynthetic rate in cotton leaves has a temperature dependence similar to the temperature dependence of the apparent  $J_{max}$ . Triose phosphate utilization by end-product synthesis may exert short-term feedback control of photosynthesis in the field under an extreme of source/sink imbalance before long-term adaptive mechanisms re-establish greater equilibrium. Anything that restricts triose phosphate utilization can limit photosynthesis (Paul and Foyer, 2001).

Photosynthetic limitations can be divided into non-stomatal limitations (including mesophyll and biochemical) and stomatal limitations (Grassi et al., 2005). At 10°C, the photosynthesis of *M. horridula* was primarily by mesophyll conductance. At 30°C, this was still true, but stomatal conductance and carboxylation efficiency had a large effect on photosynthesis. Warren and Dreyer (2006) suggested that mesophyll conductance is temperature-dependent, but this dependence varies among species (Bernacchi et al., 2002). In the present study, the plants at 20°C had higher  $g_m$  than those at 10°C and 30°C. The stomatal limitation was primarily attributable to stomatal closure (Cui et al., 2006; Zhang et al., 2001). The biochemical limitation in *M. horridula* at 30°C was mainly caused by the decrease of photosynthetic electron transport.

In conclusion, growth temperature had an important effect on photosynthetic performance. *M. horridula* grown at 20°C could obtain the highest photosynthetic rate while the photosynthetic performance at low temperature was better than at high temperature. Non-photochemical quenching was an important mechanism protecting the photosynthetic apparatus under temperature stress conditions. Although mesophyll conductance was the dominant factor limiting photosynthesis of *M. horridula* both at low and high temperature, the photosynthesis at high temperature was also limited by stomatal conductance and biochemical efficiency. The poor photosynthetic performance of *M. horridula* at high temperature might limit its cultivation at low altitudes.

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## 總狀綠絨蒿光合作用的溫度馴化

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總狀綠絨蒿 (*Meconopsis horridula* var. *racemosa* Prain.) 是世界著名的高山花卉和藏藥資源。由於難于忍受炎熱的夏季，在低海拔處栽培總狀綠絨蒿是困難的。為了評估總狀綠絨蒿在不同溫度下的光合表現及生化、氣孔和葉肉因素對其光合作用的相對限制，我們在三種溫度下研究了其光合氣體交換和葉綠素螢光。結果表明，總狀綠絨蒿植株在 20°C 下比 10°C 和 30°C 下有更高的光合能力及光化學效率，且其在低溫下 (10°C) 的光合表現要好於高溫下 (30°C)。非光化學淬滅是總狀綠絨蒿在溫度脅迫下保護光合機構的重要機制。雖然在低溫和高溫下葉肉導度都是總狀綠絨蒿的重要光合限制因素，但是在高溫下總狀綠絨蒿光合作用還受到氣孔和生化因素的限制。總狀綠絨蒿在高溫下不良的光合表現會限制其在低海拔地方的栽培。

**關鍵詞：**總狀綠絨蒿；高溫；光合作用；葉綠素螢光；光合限制。