

Impact of Simulated Warming on Growth and Floral Characteristics of Two Varieties of Medicinal Epimedium

Zhang Xuemei^{1,2*}; Fan Zengli^{1,2}; Quan Qiumei³.

¹Key Laboratory of Southwest China Wildlife Resources Conservation, China West Normal University, Ministry of Education, Nanchong, 637009, P. R. China; ² College of Life Sciences, China West Normal University, 637009, China; ³College of environmental science and engineering, China West Normal University, 637009, China.

ABSTRACT

An open top chamber (OTC) warming system was established in natural field conditions to study the impact of simulated warming on growth and floral characteristics of *E. wushanense* and *E. acuminatum*. **Results:** plant height and leaf growth were affected significantly. In +2°C warming condition, increment of plant height, leaf length and width and daily mean increment of the two varieties were significantly greater than those of the control group; however, in warming +5°C condition, the increments were significantly lower than those of the control group. Floral differentiation was presented when different treatment was adopted. Floral quantitative character of *E. acuminatum* increased markedly after +2°C warming, but declined markedly after +5°C warming; however, floral quantitative characters of *E. wushanense* declined after +2°C and +5°C warming. The results can be used as a reference for cultivation and introduction of the two varieties.

Keywords: simulated warming; *E. wushanense*; *E. acuminatum*; floral characteristics; growth status

* Authors for correspondence: ashraf@ums.edu.my

Temperature determines biological and chemical reaction rate and regulates energy, water and nutrient flow in ecosystem (Shi et al., 2009). It is a vital condition for plant growth and development, because all creatures in life process cannot do without effective accumulated temperature, and their growth is affected by temperature change (Rustad et al., 2001; Jonasson et al., 1999). Scientists conducted abundant monitoring and simulation studies and found that the global greenhouse effect beginning from the 20th century has been expanding, and mean surface temperature has risen by 0.6°C over the past 100 years (Yin et al., 2008). It has been predicted by the climate model that by the end of the 21st century, global mean surface temperature will rise by 1.6-6.4°C (IPCC, 2007). At present, the impact of climate warming on terrestrial ecosystem has become a focused concern of the ecologists at home and abroad (Usami et al., 2001).

E. wushanense, with the highest potential of development, has aroused great attention of scholars at home and abroad and already become an important medicinal plant over the years (Li et al., 2005). *E. acuminatum* is a main source of epimedium herbs in Guizhou, Chongqing, Sichuan, Guangxi, Hubei and Jiangxi, etc. (Pei and Guo, 2007) as is recorded in Ethnodrug Standards for Traditional Chinese Medicinal Materials in Guizhou (2003 edition). Current researches about the two kinds of medicinal plants are mainly concentrated in pharmacology and flowering phenology, etc. Because of excessive picking and long-term unreasonable use, wild epimedium resources are increasingly exhausted. To protect the precious resources and for the sake of further rational development and utilization, ecological study becomes quite necessary (Quan and Li, 2013).

In this research, OTC simulated warming, a popular approach of ITEX, was adopted to study the impact of simulated warming on growth and floral characteristics of *E. wushanense* and *E. acuminatum*, aiming to provide theoretical basis for further development and reasonable utilization. As global warming will inevitably change the external environment condition for plant growth and development, we can better analyze and

predict the impact of climate warming on ecosystem based on the field simulated warming experiment and the study about the response of plant growth to temperature changes, which is of great guiding significance for protecting the environment and biodiversity and maintaining stable and sustainable development of the ecosystem.

MATERIALS AND METHODS

Sample area and experimental field

Jinchengshan forest park, at the junction of Nanchong and Guang'an City in the central Sichuan Basin, is the original habitat of *E. wushanense*. It belongs to the northern subtropical region and enjoys a subtropical warm and typical monsoon climate, with four distinctive seasons and abundant heat. Biocoenosis structure of original habitat of the transplanted *E. wushanense*: white oak of fagaceae and cedarwood of cupressaceae, dominant varieties of the tree layer. Azaleas of ericaceae, Chinese mahonia of berberidaceae and *A. trifoliatum* of araliaceae, dominant varieties of the shrub layer; iris, *Pilea notata*, Indian blackberry, mugwort, etc., dominant varieties of the herb layer.

Original habitat of the transplanted *E. acuminatum* is located in Mount Emei Scenic Spot, which belongs to the subtropical moist climate zone, and enjoys abundant cloud, mist and rainfall, but little sunshine. Biocoenosis structure of habitat: theaceae, lauraceae, rosaceae, moraceae and cornaceae, etc. of the tree layer, theaceae and ericaceae of the understory shrubs, araliaceae, saxifrage, caprifoliaceae of the shrub layer, and ferns such as carex, impatiens, begonia, big leaf *Pilea notata*, rhizoma *Arisaematis* of the herbosa.

E. wushanense and *E. acuminatum* introduced in this research were grown in experimental base of School of Life Science, China West Normal University, where purple soil is predominant. The region enjoys subtropical humid monsoon climate, with an annual average rainfall of about 980-1150 mm. Other environmental factors of the three regions are shown in Table 1.

Table1 The basic environmental factors of in different habitats

Environmental factors	The habitat		
	The mountain of Jincheng temple	The mountain of Emei pingding	The experimental base of China West Normol University
Latitude and longitude	N30°45', E106°28'	N29°36',E103°21'	N 30°45',E 106°28'
Altitude (m)	714	1026	300
Air humidity (%)	64.21±2.37b	74.36±2.86a	50.48±1.41c
Light intensity (Lx)	56.28±5.05b	53.11±2.86b	63.11±1.81a
The temperature (°C)	16.12±0.15b	10.02±0.25c	18.46±0.58a
Soil moisture content(%)	16.23±0.42c	23.12±0.34b	35.12±0.45a
Soil organic matter content (%)	3.28±0.22c	10.59±0.24b	15.59±0.19a
Soil PH	4.67±0.55b	5.69±0.11a	4.66±0.45b

Note: Different letters in same column indicate the significant difference in One-way ANOVA ($p=0.05$).

RESEARCH METHODS

10 samples of *E. wushanense* and *E. acuminatum* received field and OTCs warming treatment, respectively. In the growing, flowering and fruiting periods (between March 15 and May 17, 2012), height of plant, length and width of leaf in the mid-part of plant were measured every 4d with Vernier caliper and flexible rule, and the data obtained were analyzed by SPSS19.0 and recorded in Microsoft Excel 2007.

RESULTS

Impact of warming on plant height and leaf growth rate of *E. wushanense*

Response of plant height to warming

As shown in Figure 1, plant heights of the *E. wushanense* in three different temperatures present linear growth, and plants in +2°C warming condition are higher than those in the control group C (no warming) and in +5°C warming condition. The results indicate that warming significantly affects plant height, and moderate warming is beneficial to plant growth.

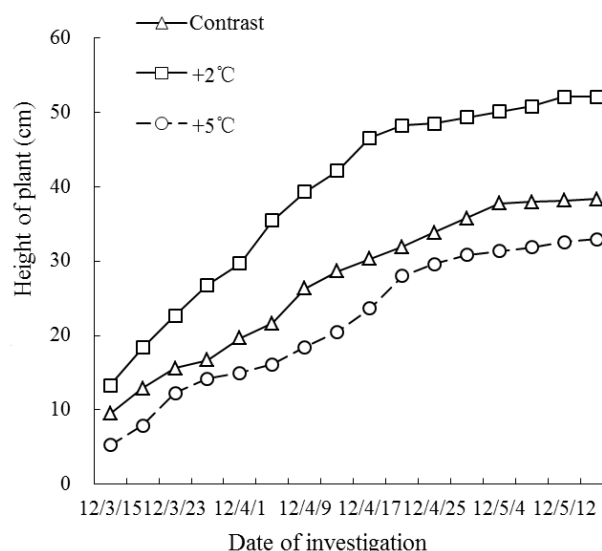


Fig. 1 The growth dynamics of height of *E. wushanense* under warming conditions

Response of leaf length and width to warming

As shown in Figure 2, leaf length growth presents a rise trend in the three warming conditions, the fastest in the growing period (April 12, 2012), and showing mild change in the breeding and the fruiting period; and growth gap among the three conditions increases gradually. Leaf length and width of *E. wushanense* in + 2°C warming condition are greater than those of the control C and in +5°C warming condition. The results show that moderate warming increase the leaf length and width.

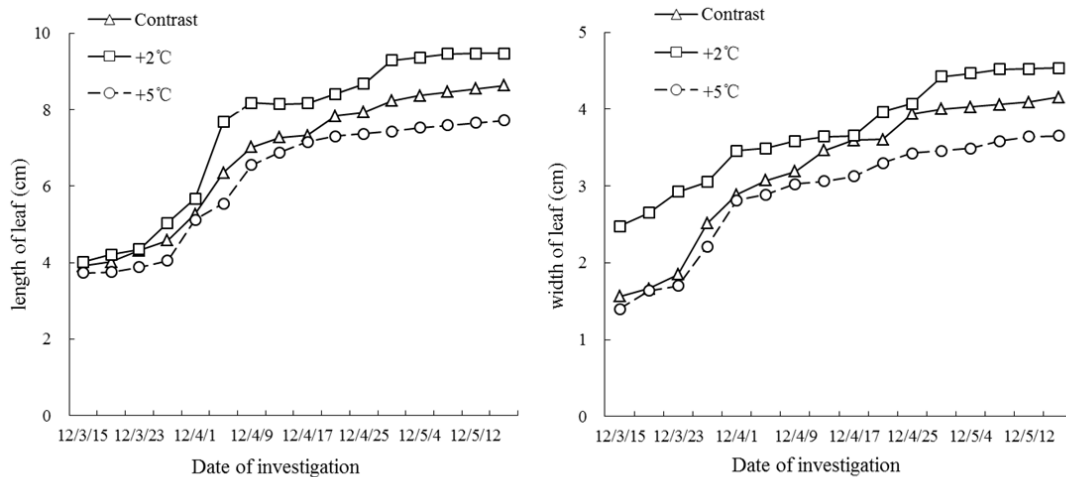


Fig.2 The growth dynamics of leaf length and width of *E. wushanense* under warming conditions

Impact of warming on plant height and leaf growth of the introduced *E. acuminatum*

Response of plant height to warming

As shown in Figure 3, plant height presents a linear growth in three different conditions, height of plants in +2°C warming condition is greater than those of the control C and in +5°C warming condition; the increase in control C presents a gentle rise trend, and height increase of plants in +5°C warming condition is relatively slow. The results are consistent with the height increase of *E. wushanense*.

Response of leaf length and width to warming

As shown in Figure 4, leaf growth presents a rise trend in the three warming conditions, the fastest in the growing period (April 5, 2012), and showing mild change in the breeding and the fruiting period; and the growth in 2°C warming condition gradually become greater than those of the control C and in +5°C warming condition. Growth rates of leaf width of plants in the three conditions are consistent in the growing period; however, in the flowering and fruiting periods, the rate in 2°C warming condition becomes larger than those in the control C and +5°C warming condition.

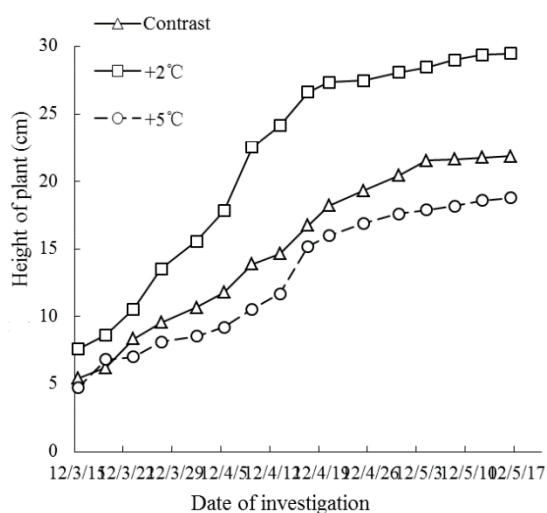


Fig.3 The growth dynamics of height of *E. acuminatum* under warming conditions

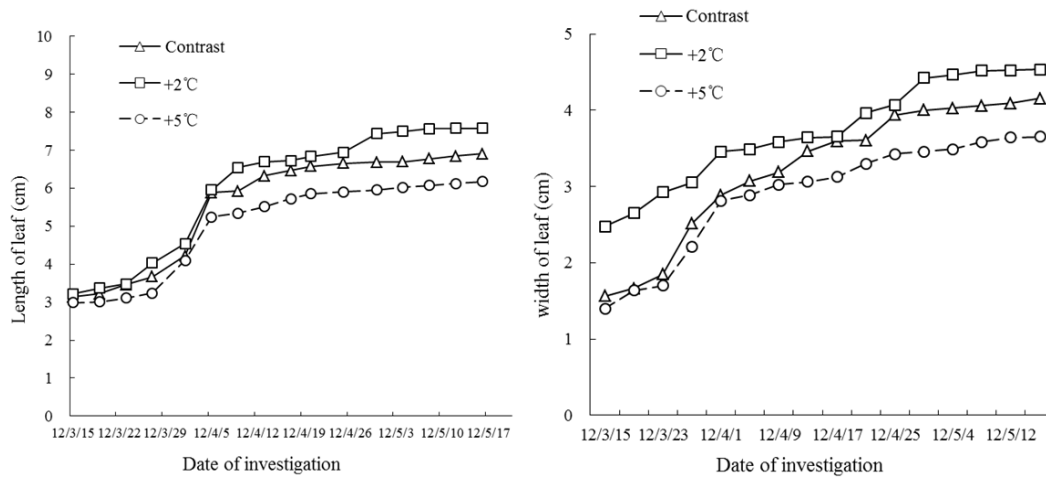


Fig.4 The growth dynamics of leaf length and width of *E.acuminatum* under warming conditions

Growth rate comparison between the two species of *Epimedium* in warming conditions

As shown in Figure 5, average daily height growth of *E. wushanense* in +2°C warming condition is significantly greater than that in concurrent control, +5°C warming condition and all treatment for *E. acuminatum*; and average daily height growth of *E. acuminatum* in +2°C warming condition is significantly greater than that in the control group and +2°C warming condition ($F_{(1,10)}=19.997$, $P=0.000$), specifically, *E. wushanense* in +2°C (0.645 ± 0.001 cm) > *E. wushanense* in control C (0.479 ± 0.004 cm) > *E. wushanense* in +5°C (0.410 ± 0.001 cm) > *E. acuminatum* in +2°C (0.368 ± 0.001 cm) > *E. acuminatum* in control C (0.274 ± 0.006 cm) > *E. acuminatum* in +5°C (0.234 ± 0.003 cm); as for growth rate of leaf length, average daily height growth of *E. wushanense* in +2°C warming condition is significantly greater than those in other groups, *E. acuminatum* in +5°C is the smallest ($F_{(1,10)}=8.715$, $P=0.001$); as for growth rate of leaf width, average daily growth of *E. wushanense* and *E. acuminatum* in +2°C is significantly greater those in other groups.

Impact of warming conditions on floral characteristics of *E. wushanense* and *E. acuminatum*

Experimental results as shown in Table 2 and Table 3: as for floral characteristics of *E. wushanense* and *E. acuminatum*, differentiation is presented when different treatments are adopted. Corolla length of *E. wushanense* in all conditions is significantly greater than that of *E. acuminatum*

in all conditions, among corolla of *E. wushanense* in control C is the longest, those in +2°C and +5°C condition the secondary, the difference is not significant; corolla of *E. acuminatum* in +5°C condition is the shortest. Consistent difference is detected in length and width of inner sepal of two species of *Epimedium* in different conditions, length and width of inner sepal of *E. acuminatum* in +2°C condition are significantly greater than those in C and +5°C conditions; length and width of inner sepal of *E. wushanense* in C and +2°C conditions are the greatest and there is no significant difference; as for the length of diagonal, *E. wushanense* in control C > *E. wushanense* in +2°C > *E. wushanense* in +5°C, *E. acuminatum* in control C > *E. acuminatum* in +2°C > *E. acuminatum* in +5°C. Length of spur of *E. wushanense* in different conditions show no significant difference and Length of spur of *E. acuminatum* in C and +2°C condition are significantly greater than that in +5°C condition; width of spur of *E. wushanense* decreases with the temperature rise, and width of spur of *E. acuminatum* in control C and +2°C are greater than that in +5°C; stamen length and filament length of *E. acuminatum* in +2°C condition are significantly greater than those in C and +5°C conditions, and stamen length and filament length of *E. wushanense* in control C are significantly greater than those in +2°C and C +5°C warming conditions. Stigma length of *E. acuminatum* among different conditions show no significant difference, while stigma length of *E. wushanense* in control C and +2°C are significantly greater than that in +5°C. As for ovary length and ovary

diameter, the two varieties follow the same trend, and the values in control C and +2°C are significantly greater than those in +5°C. Overall, floral quantitative character of *E. acuminatum* after +2°C warming rises significantly, and falls

after +5°C warming; floral quantitative character of *E. wushanense* falls after +2°C and +5°C warming.

Table 2 Comparison of floral department in *E. acuminatum* under warming conditions

Content	Contrast	+2°C	+5°C
Length of corolla(cm)	35.430±2.411a	33.853±1.713a	26.348±0.938b
Length of inner sepal(cm)	13.512±0.587b	15.132±0.208a	12.968±0.309b
Width of inner sepal(cm)	6.188±0.528b	7.877±0.550a	5.745±0.291b
Length diagonal of spur(cm)	34.304±1.824a	33.763±1.803a	25.508±0.811b
Length of spur(cm)	13.455±1.305a	14.682±1.426a	9.357±0.496b
Width of spur(cm)	3.432±0.185ab	3.500±0.187a	3.375±0.116b
Length of filament(cm)	5.378±0.116b	5.800±0.074a	5.513±0.080ab
Length of filament(cm)	4.038±0.091b	4.622±0.055a	4.092±0.054b
Length of style(cm)	2.910±0.140a	2.932±0.131a	2.915±0.113a
Length of pistil(cm)	3.014±0.240a	3.093±0.098a	2.702±0.114b
Diameter of pistil(cm)	0.864±0.045a	0.817±0.021a	0.778±0.021b

Note: Different lowercase letters in the same row indicate significant differences ($P < 0.05$). Data for average \pm standard error (n = 6).

Table 3 Contrast of floral department in *E. wushanense* under warming conditions

Content	Contrast	+2°C	+5°C
Length of corolla(cm)	49.983±1.599a	43.353±2.251b	39.452±1.578c
Length of inner sepal(cm)	7.912±0.283a	7.262±0.333b	6.317±0.113c
Width of inner sepal(cm)	3.643±0.179a	3.275±0.089b	3.099±0.084bc
Length diagonal of spur(cm)	49.761±1.594a	41.573±3.100b	39.499±1.091c
Length of spur(cm)	21.734±0.956a	19.890±0.882b	19.599±1.147b
Width of spur(cm)	4.014±0.185a	3.490±0.105b	3.016±0.058c
Length of filament(cm)	5.821±0.174a	4.862±0.127b	4.663±0.053b
Length of filament(cm)	4.246±0.137a	3.848±0.094b	3.675±0.037b
Length of style(cm)	2.716±0.099b	2.443±0.140c	2.969±0.146a
Length of pistil(cm)	2.839±0.130a	2.482±0.095b	2.386±0.090b
Diameter of pistil(cm)	0.906±0.021a	0.818±0.018b	0.796±0.023bc

Note: Different small letters mean significant level 0.05. Data for average \pm standard error (n = 6).

DISCUSSIONS

Many studies indicate that warming can promote plant growth and biomass accumulation. Morphological characteristics of plants play an important role in the process of their environment adaptation (Danby and Hik, 2007; Sandvik S M et al., 2004; Welker et al., 1997; Yin et al., 2008). Based on appropriate morphological adjustment, plants may possess and absorb more resources, and become more advantageous in growth and competition (Gielen et al., 2002). Growth of over-ground part of plants affects light energy acquisition and moisture absorption. Temperature change affects plant growth often by changing the temperature of plant root. It has been found that 1°C ground temperature change can cause great change in plant growth and nutrient absorption, and the response of leaf growth to root

temperature is the most obvious (Walker, 1969; Feng and Sun, 1995). Hobbie interprets Ploygonum's research that warming causes increase in dry weight and length of leaves, which is further verified by the parameters of *E. wushanense* and *E. acuminatum* obtained in this research, such as changes in plant height, leaf length and width (Hobbie and Chapin, 1998). In +2°C warming condition, growth of the two varieties of epimedium in plant height, leaf length and width is greater than that in control C and 5°C warming condition, and the daily average growth in plant height, leaf length and width is significantly greater than that in control C and +5°C condition, suggesting that moderate temperature can promote the growth of *E. wushanense* and *E. acuminatum*.

Impact of climate warming on plant biomass and nutrient depends on whether the temperature

approximates or exceeds the optimal temperature for plant growth. Change of morphological characteristics of plants under different warming conditions is an important index reflecting the range of temperature resistance of plants. Based on comparing the increments of plant height, leaf length and width of the two varieties, *E. wushanense* is found to be the most sensitive to temperature. Growth of the two varieties in +5°C warming condition is unsatisfactory, suggesting that high temperature leads to poor performance of growth. Tjoelker studies warming effect on several varieties of trees and found that conifers such as larch and picea glauca had the highest relative growth rate in moderate temperature condition, while broadleaf varieties such as *Populus tremuloides* and *Betula papyrifera* had the highest

relative growth rate in high temperature condition (Tjoelker et al., 1998). The experimental results suggest that 2°C temperature rise significantly increased plant height, leaf length and width of *E. wushanense* and *E. acuminatum* (Figure 5), i.e. light energy acquisition and utilization both in altitudinal and width range, and assimilation were enhanced, thus competitiveness was improved and living space is enlarged. Under the condition of environmental stress or limited resources, plants allocate more biomass to leaves to capture light energy more effectively; after the growing period, however, three methods maintained steady growth rate. It may be because plants in the flowering period need more nutrition for propagation, thus resulting low rate of growth.

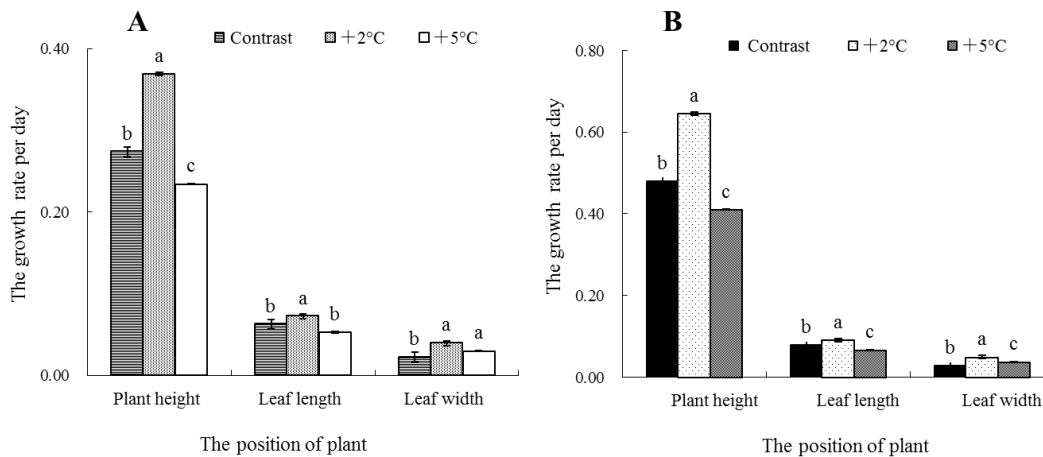


Fig.5 Dynamics of leaf area growth in two *Epimedium* species under warming conditions
Note: Fig (A) is *E. acuminatum*, (B) is *E. wushanense*

In addition, optimum temperature of different parts or organs of a variety also differs. Wada conducted short-term artificial warming experiment against *Geum pentapetalum* and found that its reproductive organs are more sensitive than vegetative organs (Wada et al., 1998). Kudo and Suzuki studied the effect of OTC warming on 5 varieties of plants and showed that warming response of nutritional component and reproductive component of different varieties also differs (Kudo and Suzuki, 2003). Similar results were obtained in this research: for *E. acuminatum*, floral quantitative character was significantly increased in +2°C warming, while decreased in +5°C warming; for *E. wushanense*, the effect is $C \geq +2^\circ C > +5^\circ C$. Thus, global warming affects floral quantitative character of *E. acuminatum* and *E. wushanense*. Floral characteristics are closely associated with pollinator behavior, pollination

mechanism as well as mating system and successful reproduction of plants, etc., so if a floral characteristic can significantly increase fitness of the plant, the character is selected by a particular pollinator or functional group of pollination (Barrett, 1998; Li et al., 2013). Whether the change in floral quantitative character caused by climate change can affect pollination process of *E. wushanense* and *E. acuminatum* will be studied further.

In conclusion, epimedium grown in different climate regions have different adaptability in new environment and different warming response. In the condition of moderate warming, the transplanted *E. acuminatum*, whose light energy acquisition and utilization both in altitudinal and width range were enhanced, obtained higher competitiveness and greater living space, while *E. wushanense* was given no warming or +2°C

warming treatment. These results can be used as a reference for cultivation and introduction of the two varieties.

Acknowledgements This research was financially supported by grants from China West Normal University (Grant Nos. 412565; Grant Nos. 412532).

REFERENCE

- Danby R K, Hik D S. Responses of white spruce (*Picea glauca*) to experimental warming at a subarctic alpine treeline. *Global Change Biology*, 2007,13:437-451.
- Domisch T, Finér L, Lehto T. Effect of soil temperature on nutrient allocation and mycorrhizas in Scots pine seedlings. *Plant and Soil*, 2002, 239:173-185.
- IPCC (Intergovernmental Panel on Climate Change) (2007). Contribution of working group III to the fourth assessment report of the intergovernmental panel on climate change. In: Metz B, Davidson OR, Bosch PR, Dave R, Meyer LA eds. *Climate Change in 2007: Mitigation*. Cambridge University Press, Cambridge, UK.
- Rustad L E, Campbell J L, Marion G M, Norby R J, Mitchell M J, Hartley A E, Cornelissen J H C, Gurevitch J, Gcte-News. A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. *Oecologia*, 2001,126(4): 543-562.
- Jonasson S, Michelsen A, Schmidt I K, Nielsen E V. Responses in microbes and plants to changed temperature, nutrient, and light regimes in the Arctic. *Ecology*, 1999,80(6): 1828-1843.
- Sandvik S M, Heegaard E, Elven R et al. Responses of alpine snow bed vegetation to long term experimental warming. *Ecoscience*, 2004, 11:150-159.
- Welker J M, Molau U, Parsons A N et al. Responses of dryas octopetala to ITEX environment manipulations: A synthesis with circumpolar comparisons. *Global Change Biology*, 1997, 3: 61-73.
- Usami T, Lee J, Oikawa T. Interactive effects of increased temperature and CO² on the growth of *Quercus myrsinaefolia* saplings. *Plant, Cell and Environment*, 2001,24: 1007-1019.
- PEI Li kuan, GUO Bao lin. A review on research of raw material and cut crude drug of *Herba Epimedium* in last ten years, *China Journal of Chinese Materia Medica*, 2007, 32(6): 466-471.
- YIN HuaJun, LAI Ting, CHENG XinYing, JIANG XianMin, LIU Qing. Warming Effects on Growth and Physiology of Seedlings of *Betula albo-sinensis* and *Abies faxoniana* under Two contrasting Light Conditions in Subalpine Coniferous Forest of Western Sichuan, China. *Journal of Plant Ecology (Chinese Version)*, 2008, 32(5): 1072~1083.
- LI Zuo Zhou, XU Yanqin, WANG Ying, HUANG Hongwen. Status and prospect of research on medicinal plants of *Epimedium* L. *Chinese Traditional and Herbal Drugs*. 2005. 36(2): 289-296.
- QUAN Qiumei, LI Yunxiang. Flowering phenology and reproductive feature of *E. wushanense*. *Chinese Journal of Ecology*, 2013,32(4): 859-866.
- SHI Fusun, WU Ning, WU Yan, WANG Qian. Effect of Simulated Temperature Enhancement on Growth and Photosynthesis of *Deschampsia caespitosa* and *Thlaspi arvense* in Northwestern Sichuan, China. *Chin J Appl Environ Biol*, 2009,15(6): 750~755.

Tjoelker M G, Oleksyn J, Reich P B. Seedlings of five boreal tree species differ in acclimation of net photosynthesis to elevated CO₂ and temperature. *Tree physiology*, 1998, 18(11): 715-726.

Received: June 15, 2016;
Accepted: June 24, 2016

Walker J M. One-degree increments in soil temperatures affect maize seedling behavior[J]. *Soil Science Society of America Journal*, 1969, 33(5): 729-736.

Feng Yulong, Sun Guobin. Influence of temperature of root system on plant(I)-influence of root temperature on plant growth and photosynthesis. *Journal of Northeast Forestry University*. 1995, 23(3): 63-69.

Hobbie S E, Chapin III F S. The response of tundra plant biomass, aboveground production, nitrogen, and CO₂ flux to experimental warming. *Ecology*, 1998, 79(5): 1526-1544.

Gielen B, Calfapietra C., Claus A. Crown architecture of *Populus* spp. Was differentially modified by free-air CO₂ enrichment (POPFACE). *New Phytologist*, 2002, 53(1): 91-99.

Wada N, Miyamoto M, Kojima S. Responses of reproductive traits to short-term artificial warming in a deciduous alpine shrub *Geum pentapetalum* (Rosaceae) Proceedings of the NIPR Symposium on Polar Biology. National institute of polar research, 1998: 137-146.

Li X.X., Wang IL, Gituru R.W., Guo Y.H.' Yang C.F. Pollen packaging and dispensing: Adaption of patterns of anther dehiscence and flowering traits to pollination in three *Epimedium* species. *Plant Biology*, 2014, 16: 227-233.

Barrett S C H. The evolution of mating strategies in flowering plants. *Trends in Plant Science*. 1998. 3: 335-341

Kudo G, Suzuki S. Warming effects on growth, production, and vegetation structure of alpine shrubs: a five-year experiment in northern Japan. *Oecologia*, 2003, 135(2): 280-287.