

Research paper

Leaf physiological and anatomical responses of two sympatric *Paphiopedilum* species to temperature

Jing-Qiu Feng ^{a, b}, Ji-Hua Wang ^c, Shi-Bao Zhang ^{a, *}^a Key Laboratory of Economic Plants and Biotechnology, Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650201, Yunnan, China^b University of Chinese Academy of Sciences, Beijing, 100049, China^c Flower Research Institute of Yunnan Academy of Agricultural Sciences, Kunming, 650205, Yunnan, China

ARTICLE INFO

Article history:

Received 28 December 2020

Received in revised form

19 March 2021

Accepted 5 May 2021

Available online 17 May 2021

Keywords:

Conservation

Cultivation

High-temperature

Leaf anatomy

Paphiopedilum

Photosynthesis

ABSTRACT

Paphiopedilum dianthum and *P. micranthum* are two endangered orchid species, with high ornamental and conservation values. They are sympatric species, but their leaf anatomical traits and flowering period have significant differences. However, it is unclear whether the differences in leaf structure of the two species will affect their adaptabilities to temperature. Here, we investigated the leaf photosynthetic, anatomical, and flowering traits of these two species at three sites with different temperatures (Kunming, 16.7 ± 0.2 °C; Puer, 17.7 ± 0.2 °C; Menglun, 23.3 ± 0.2 °C) in southwest China. Compared with those at Puer and Kunming, the values of light-saturated photosynthetic rate (P_{max}), stomatal conductance (g_s), leaf thickness (LT), and stomatal density (SD) in both species were lower at Menglun. The values of P_{max} , g_s , LT, adaxial cuticle thickness (CT_{ad}) and SD in *P. dianthum* were higher than those of *P. micranthum* at the three sites. Compared with *P. dianthum*, there were no flowering plants of *P. micranthum* at Menglun. These results indicated that both species were less resistance to high temperature, and *P. dianthum* had a stronger adaptability to high-temperature than *P. micranthum*. Our findings can provide valuable information for the conservation and cultivation of *Paphiopedilum* species.

Copyright © 2021 Kunming Institute of Botany, Chinese Academy of Sciences. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co., Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Members of the genus *Paphiopedilum* (Orchidaceae) are world-famous ornamental orchids because of their unique flower shape and colors, large flowers, and long flower lifespan. They have been cultivated throughout the world for more than 100 years, but their commercial production is still limited (Averyanov et al., 2003; Liu et al., 2009). One of the important reasons for this is the insufficient understanding of the environmental adaptability of *Paphiopedilum* species, which limits the development of cultivation techniques. Meanwhile, due to illegal collection and habitat destruction, the number of wild populations and individuals of *Paphiopedilum* have drastically decreased (Liu et al., 2009). *Paphiopedilum* species are sensitive to environmental change. For example, the forest exploitation influences the light intensity and temperature in the natural distribution areas of *Paphiopedilum*

(Averyanov et al., 2003). They are a particularly vulnerable group of plants which are amongst the first to disappear during degradation of primary native habitats. All known *Paphiopedilum* species are listed in Appendix I of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2012), and are strictly protected by this international convention. Both the cultivation and conservation of *Paphiopedilum* species depend on knowledge of their environmental adaptabilities. However, only a few studies have focused on the adaptations of *Paphiopedilum* species to low temperature and light intensity (Zhang et al., 2011; Yang et al., 2017), and their environmental requirements and adaptive mechanisms remain unclear.

The genus *Paphiopedilum* comprises more than 70 species, which usually occur in limestone or mountainous forests of tropical and subtropical zones from Asia to the Pacific Islands (Cribb, 1998; Liu et al., 2009). Previous studies have suggested that *Paphiopedilum* plants are characterized by drought tolerance derived from their leaf anatomical traits, including fleshy leaves, thick leaves, thick cuticles, bigger adaxial epidermal cells, lower total stomata area and sunken stomata (Guan et al., 2011; Zhang et al., 2011; Yang et al., 2018). Stomatal and vein densities also tend to increase from terrestrial to

* Corresponding author.

E-mail address: sbzhang@mail.kib.ac.cn (S.-B. Zhang).

Peer review under responsibility of Editorial Office of Plant Diversity.

facultative and epiphytic *Paphiopedilum* species (Zhang et al., 2012). The lack of guard cell chloroplasts affects the induction of photosynthesis in *Paphiopedilum*, which may reflect a physiological adaptation to water shortage (Assmann and Zeiger, 1985; Zhang et al., 2011). Therefore, previous studies infer that leaf morphological traits play an important function in reducing the loss of water (Guan et al., 2011; Zhang et al., 2011). However, these species vary largely in their growing environments, leaf morphology and anatomy, flowering period and floral lifespan (Guan et al., 2011; Zhang et al., 2012, 2017). Research on how leaf morphology and anatomy affect environmental adaptations of *Paphiopedilum* species through controlled experiments is still lacking.

Photosynthesis is a major mechanism for plants to assimilate carbon, and is extremely sensitive to environmental changes, such as light, temperature, and water (Long et al., 2006; Greer and Weedon, 2011; Toscano et al., 2018). Thus, the photosynthetic rate is a crucial trait reflecting a plant's response to environmental changes. Usually, plants have a higher light-saturated photosynthetic rate under optimum temperature conditions (An et al., 2017). Both low and high temperatures can depress the photosynthetic rate and stomatal conductance of plants (Greer and Weston, 2010; Greer and Weedon, 2011; Wu et al., 2014). Low temperature can decrease photosynthetic performance of *Phalaenopsis* plants (Pollet et al., 2009). Similarly, high temperature reduces relative growth rates by the direct effects of temperature on photosynthesis (Marchin et al., 2014). The photosynthetic rate is depressed, while respiration is promoted when plants are exposed to a temperature which is above the photosynthetic optimum. The vegetative growth is strongly inhibited due to the imbalance between carbon-fixation and consumption (Iersel, 2003). Recently, Yang et al. (2017) found that *P. armeniacum* and *P. micranthum* can use cyclic electron flow to protect photosystem I and photosystem II under chilling conditions. However, how the photosynthesis of *Paphiopedilum* plants respond to temperature changes remains unclear.

Leaf morphology and anatomy play an important role in response to changing environments, because they affect light capture, CO₂ diffusion conductance, heat balance, water loss and storage of leaves. Leaf anatomical traits are significantly influenced by temperature. Plants will increase their leaf thicknesses under warming conditions (Soudzilovskaia et al., 2013). Under high temperatures, plants may increase their leaf and adaxial cuticle thickness (Zheng et al., 2013; Zhou et al., 2019; Habermann et al., 2019), but decrease stomatal density (Ferris et al., 1996). Recently, Wu et al. (2018) found that stomatal density of *Schima superba* significantly decreases under warming conditions, while a non-significant change is observed in *Syzygium rehderianum*. Warm temperatures in the early spring, when shoots are emerging, appear favored, while high temperatures during a thesis appear detrimental, reducing both vegetative growth and flowering (Bleho et al., 2021). However, how the leaf anatomy of *Paphiopedilum* plants varies with the change of temperature remains unclear.

Phenotypic plasticity may play an important role in the establishment of plant population in novel environments, either after transplant to a new habitat or as a response to changing environments (Richards et al., 2006; Matesanz et al., 2010). Although the ability to respond to environmental change is often beneficial, phenotypic plasticity, just like any other aspect of the phenotype, cannot automatically be assumed to be adaptive (Richards et al., 2006). It is adaptive plasticity, however, that is of particular importance for ecological and evolutionary studies (Richards et al., 2006). For example, compared with stomatal length, stomatal density is relatively plastic (Richardson et al., 2001) and potentially adaptive to environmental change (Poulos et al., 2007; Sekiya and Yano, 2008). Thus, we speculate that large phenotypic plasticity indices of *Paphiopedilum* species may help them to tolerate the change of temperature.

In the present study, we investigated the responses of leaf photosynthetic and anatomical traits of *P. dianthum* and *P. micranthum* to different environmental temperatures. These two *Paphiopedilum* species can occur in the same natural habitats, but *P. dianthum* plants have a greater leaf, cuticle and epidermis thickness, and higher stomata density than *P. micranthum* (Zhang et al., 2012; Yang et al., 2018). In addition, *P. dianthum* flowers in autumn, while *P. micranthum* flowers in spring. This indicates that there may be differences in temperature adaptation between the two species. Thus, our aim was to address how do photosynthetic, anatomical traits and flowering performances of two sympatric species with different leaf anatomies respond to different temperatures. Such information will contribute to the conservation and cultivation of *Paphiopedilum* species. We speculate that the adaptation of the two *Paphiopedilum* species to temperature is related to their geographical distribution, and the plasticity of leaf anatomical traits may play an important role in the adaptations of two *Paphiopedilum* species to growth temperature.

2. Materials and methods

2.1. Study sites and plant materials

The study was carried out at Menglun, Puer, and Kunming in southwest China (Fig. 1). These three sites locate in tropical, south subtropical, and mid-subtropical zone, respectively. The daily average temperature and air relative humidity was 23.3 ± 0.2 °C and 86% at Menglun, 17.7 ± 0.2 °C and 81% at Puer, and 16.7 ± 0.2 °C, and 73% at Kunming, respectively (Fig. 2).

In this study, two sympatric species, *P. dianthum* and *P. micranthum*, were chosen for the experiments. *P. dianthum* occurs on rocks in evergreen broad-leaved forests at an elevation of 1000–2250 m. *P. micranthum* occurs in rocky and bushy places or crevices of rocks in forests in limestone areas at an elevation 400–1700 m (Liu et al., 2009). In November 2018, two-year old artificially propagated plants (300 plants of each species at each site) were placed at three study sites. The plants were planted in porous plastic pots (10 cm × 15 cm) filled with bark and humus (7/3, v/v). We used 70% shade-net to keep the maximum light intensity at approximately $400\text{--}600 \mu\text{mol m}^{-2} \text{s}^{-1}$. These plants were watered as

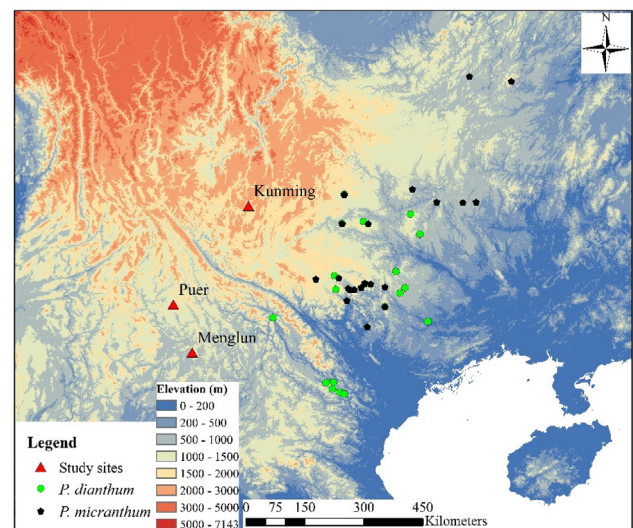


Fig. 1. The study sites (red triangles) and the natural distribution of *Paphiopedilum dianthum* (green circles) and *P. micranthum* (dark pentagons). The map was made with a software of ArcGIS (Version 10.2, Esri, Inc., CA, USA).

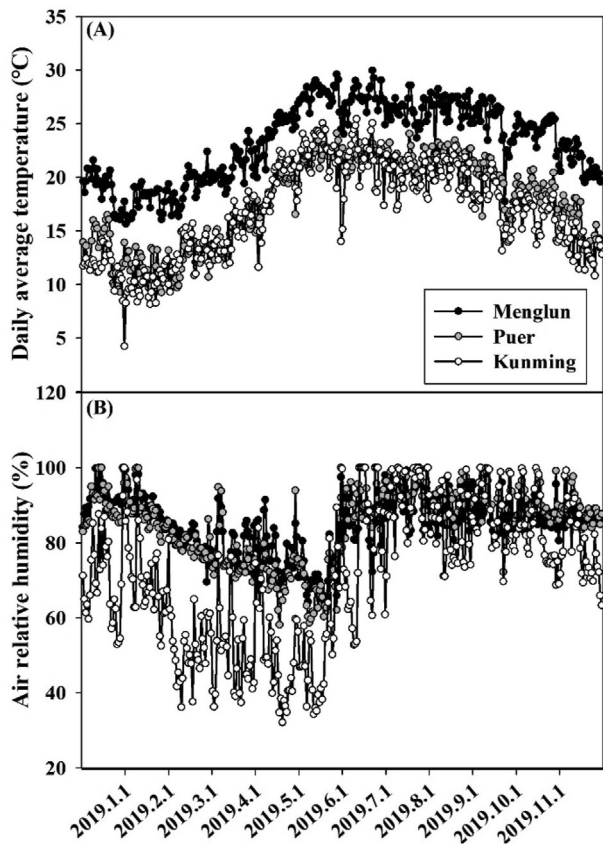


Fig. 2. Change in daily average temperature (A) and air relative humidity (B) at the three study sites.

needed, and fertilized with control release fertilizer (Osmocote, nitrogen: phosphate: potash = 14:14:14, Geldermalsen, Netherlands) about 0.5 g in each pot every month.

2.2. Measurement of photosynthetic gas exchange

All measurements of leaf gas exchange were performed in an open infrared gas exchange system with an integrated fluorescence chamber (LI-6400-40; Li-Cor, Lincoln, NE, USA) in June and December 2019. Before photosynthetic measurements, the leaf steady-state conditions of *P. dianthum* and *P. micranthum* were induced for 20–30 min using a chamber CO_2 concentration of $400 \mu\text{mol mol}^{-1}$ and a photosynthetic photon flux density (PPFD) of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$. Photosynthetic light response curves were tested with light intensities ranging from 1000 to $0 \mu\text{mol m}^{-2} \text{s}^{-1}$. The CO_2 concentration in the chamber was set to $400 \mu\text{mol mol}^{-1}$, and temperature was 25°C . Photosynthetic rates were recorded within a steady state by waiting for 3 min at each light intensity. Five mature leaves per species were measured from different individuals. The respiration rate in dark (R_d) and light-saturated photosynthetic rate (P_{max}) were obtained during the measurements of light response curves which were fitted with the software of Photosynthesis (LI-COR Application, Lincoln, NE, USA). The stomatal conductance (g_s), transpiration rate (T_r), and instantaneous water use efficiency (WUE_i) were calculated under saturated light.

2.3. Leaf anatomical and floral traits

In December 2019, ten leaves were collected from the second leaf of different individuals from the top down per species at each

site, which were produced in the new growth environment. Of the ten leaves, five were fixed in FAA (95% ethanol: distilled water: formaldehyde: glacial acetic acid, 10:7:2:1, v/v/v/v) for at least 24 h. The other five leaves were measured with a LI-3000 A leaf portable area meter (Li-Cor, Lincoln, NE, USA), then the samples were oven-dried at 70°C to a constant weight. The leaf dry mass was then weighed and evenly divided into three samples. The leaf dry mass per unit area (LMA) was calculated by the leaf dry mass divided by the leaf area. The leaf carbon content (C_{mass}), leaf nitrogen content (N_{mass}), and leaf phosphorus content (P_{mass}) were measured using an element analyzer (Elementar Analysensysteme GmbH, Vario EL III, Hanau, Germany).

In order to calculate the stomatal density (SD) and stomatal area (A_s), about 1 cm^2 of the middle parts of the leaves were cut, and then immersed into the disinfectant to remove the leaf mesophyll and chlorophyll. The other parts of leaf were dehydrated in an ethanol series and embedded in paraffin for sectioning. Transverse sections were made on a Leica RM2126RT rotary microtome (Leica Inc., Bensheim, Germany). After treatments, five views of each abaxial epidermis were observed and photographed with a Leica DM2500 light microscope (Leica Microscope Vertrieb GmbH, Wetzlar, Germany). To measure the thickness of the leaf (LT), cuticle (CT_{ad} and CT_{ab}), epidermis (ET_{ad} and ET_{ab}) and mesophyll (MT), five sections tissue of each leaf were observed and photographed with a light microscope. All the images were analyzed with the ImageJ software package. The flower number per stalk was recorded during anthesis. The peduncle, petal, lip, and dorsal sepal size were measured with a ruler.

2.4. Statistical analysis

Statistical analyses were performed with the IBM SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA). A one-factor ANOVA (Tukey's posthoc test) was performed at $p = 0.05$ significance level to determine the differences in leaf photosynthetic, leaf anatomical, and floral traits among sites after testing for normality and homogeneity of variances. The differences between two parameters were analyzed with t tests of independent samples. The light response curves were fitted with the software of Photosynthesis (LI-COR Application). The phenotypic plasticity index was calculated for each measured variable as the difference between maximum and minimum values divided by the maximum value (Valladares et al., 2000). The map of natural distribution of *Paphiopedilum* species was performed on the software of ArcGIS 10.2 (Esri, Inc., CA, USA). Graphic images were produced using the Sigma Plot 10.0 package (Systat Software Inc., CA, USA).

3. Results

3.1. Leaf photosynthesis at different sites

The leaf photosynthetic rates (P_n) of *P. dianthum* and *P. micranthum* increased greatly with the increase in PPFDs when the light intensity below $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the three sites (Fig. 3). There were slightly changes of leaf photosynthetic rates of the two *Paphiopedilum* species with the increase in PPFDs when the light intensity over $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the three sites. Compared with Puer and Menglun, the two tested species had a higher P_{max} and g_s at Kunming (Table 1). For example, the P_{max} for *P. dianthum* at Kunming ($5.24 \pm 0.29 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was significantly higher than at Menglun ($3.24 \pm 0.32 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$). Similarly, the g_s at Kunming ($97.0 \pm 11.5 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) was also significantly higher than at Menglun ($39.4 \pm 14.4 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$). For *P. micranthum*, the P_{max} ($3.00 \pm 0.10 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) and g_s

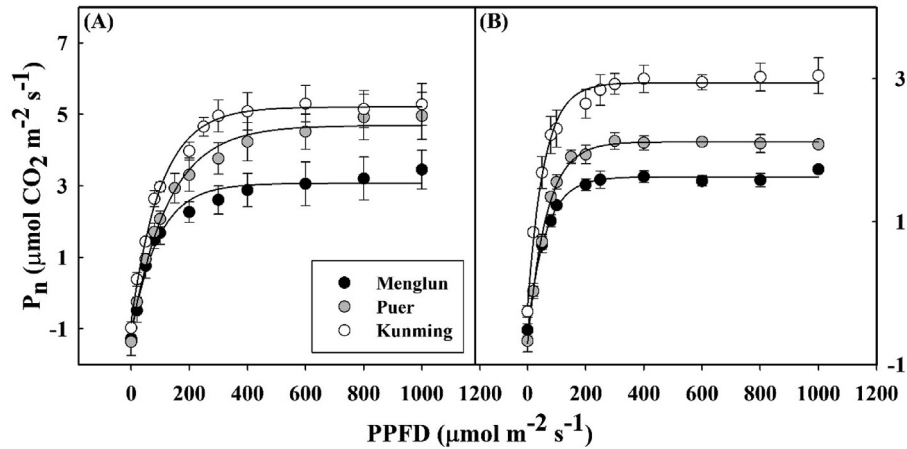


Fig. 3. Responses of photosynthetic rate (P_n) to photosynthetic photon flux density (PPFD) in *Paphiopedilum dianthum* (A) and *P. micranthum* (B) at the three study sites. The light response curve is fitted with Exponential Rise to Maximum (single, two parameters) in Sigma Plot 10 package. Each data point represents mean \pm SE for five measurements from individual plants.

Table 1
Leaf photosynthetic, anatomical and physiological traits of *Paphiopedilum dianthum* and *P. micranthum* at the three study sites.

Species	Traits	Menglun (alt. 570 m)	Puer (alt. 1302 m)	Kunming (alt. 1990 m)	Plasticity index (%)
<i>P. dianthum</i>	R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.64 \pm 0.24 ^a	0.68 \pm 0.03 ^a	0.48 \pm 0.04 ^a	29.4
	P_{max} ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	3.24 \pm 0.32 ^b	4.80 \pm 0.32 ^a	5.24 \pm 0.29^a	38.2
	g_s ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	39.4 \pm 14.4 ^b	61.7 \pm 8.0 ^{ab}	97.0 \pm 11.5^a	59.4
	T_r ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	0.74 \pm 0.17 ^a	0.54 \pm 0.10 ^a	0.71 \pm 0.07 ^a	27.0
	WUE_i ($\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$)	6.87 \pm 4.17 ^a	7.53 \pm 1.05 ^a	7.04 \pm 0.28 ^a	8.8
	LT (μm)	1043.4 \pm 19.7 ^c	1371.8 \pm 33.0^a	1223.1 \pm 18.1 ^b	23.9
	CT_{ad} (μm)	34.57 \pm 1.38 ^a	35.04 \pm 1.18 ^a	31.54 \pm 1.71 ^a	10.0
	CT_{ab} (μm)	24.35 \pm 0.89 ^a	23.27 \pm 0.70 ^a	26.19 \pm 1.20 ^a	11.1
	ET_{ad} (μm)	304.6 \pm 16.06 ^b	510.9 \pm 13.5^a	451.4 \pm 16.4 ^a	40.4
	ET_{ab} (μm)	59.42 \pm 1.75 ^a	54.45 \pm 1.42 ^a	60.70 \pm 1.79 ^a	10.3
	MT (μm)	613.9 \pm 10.1 ^b	715.4 \pm 23.8^a	659.4 \pm 16.4 ^{ab}	14.2
	SD (mm^{-2})	36.0 \pm 0.4 ^b	45.3 \pm 2.2^a	39.6 \pm 2.1 ^{ab}	20.5
	A_s (μm^{-2})	1298.6 \pm 27.6 ^a	1463.6 \pm 23.2 ^a	1477.4 \pm 29.2 ^a	12.1
	LMA (g m^{-2})	146.4 \pm 8.01 ^a	165.2 \pm 5.5 ^a	161.1 \pm 7.8 ^a	11.4
	C_{mass} (%)	49.38 \pm 0.24^a	48.30 \pm 0.38 ^{ab}	47.53 \pm 0.40 ^b	3.7
	N_{mass} (%)	1.05 \pm 0.09 ^a	0.86 \pm 0.10 ^a	1.11 \pm 0.10 ^a	22.5
	P_{mass} (%)	1.29 \pm 0.19 ^a	1.06 \pm 0.10 ^a	1.20 \pm 0.05 ^a	17.8
<i>P. micranthum</i>	R_d ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	0.26 \pm 0.04 ^a	0.33 \pm 0.08 ^a	0.13 \pm 0.04 ^a	60.6
	P_{max} ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	1.62 \pm 0.04 ^c	2.10 \pm 0.05 ^b	3.00 \pm 0.10^a	46.0
	g_s ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	31.1 \pm 5.5 ^b	46.5 \pm 3.3 ^{ab}	60.8 \pm 11.1^a	48.8
	T_r ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	0.26 \pm 0.05 ^b	0.46 \pm 0.05^a	0.35 \pm 0.04 ^{ab}	43.5
	WUE_i ($\mu\text{mol CO}_2 \text{mmol}^{-1} \text{H}_2\text{O}$)	6.87 \pm 1.64 ^a	5.91 \pm 1.23 ^a	7.67 \pm 0.74 ^a	22.9
	LT (μm)	949.8 \pm 20.0 ^b	905.2 \pm 16.7 ^b	1041.0 \pm 35.9^a	13.0
	CT_{ad} (μm)	22.05 \pm 0.83 ^b	23.45 \pm 1.04 ^{ab}	27.24 \pm 2.35^a	19.0
	CT_{ab} (μm)	19.77 \pm 0.70 ^a	18.54 \pm 1.02 ^a	19.24 \pm 1.15 ^a	6.2
	ET_{ad} (μm)	165.0 \pm 6.6 ^a	156.2 \pm 5.4 ^a	164.6 \pm 10.1 ^a	5.4
	ET_{ab} (μm)	68.02 \pm 2.69 ^a	68.87 \pm 2.64 ^a	66.57 \pm 2.09 ^a	3.3
	MT (μm)	674.2 \pm 16.1 ^b	644.0 \pm 15.3 ^b	759.42 \pm 29.1^a	15.2
	SD (mm^{-2})	16.0 \pm 0.6 ^b	19.2 \pm 1.0^a	17.6 \pm 0.6 ^{ab}	16.7
	A_s (μm^{-2})	2775.0 \pm 61.4 ^a	2318.9 \pm 44.0 ^a	2327.0 \pm 45.5 ^a	16.4
	LMA (g m^{-2})	177.4 \pm 10.6 ^a	170.1 \pm 7.6 ^a	160.4 \pm 8.4 ^a	9.6
	C_{mass} (%)	43.86 \pm 0.20 ^a	44.02 \pm 0.43 ^a	43.44 \pm 0.19 ^a	1.3
	N_{mass} (%)	0.90 \pm 0.07 ^a	0.77 \pm 0.10 ^a	0.92 \pm 0.07 ^a	16.3
	P_{mass} (%)	0.53 \pm 0.04 ^a	0.49 \pm 0.05 ^a	0.54 \pm 0.04 ^a	9.2

Different letters indicated significant differences between study sites and the maximum value was bold ($P < 0.05$, based on ANOVA, followed by Tukey's tests for comparison). Values were means \pm SE ($n = 5$). R_d , respiration rate in dark; P_{max} , light-saturated photosynthetic rate; g_s , stomatal conductance; T_r , transpiration rate; WUE_i , instantaneous water use efficiency; LT, leaf thickness; CT_{ad} , adaxial cuticle thickness; CT_{ab} , abaxial cuticle thickness; ET_{ad} , adaxial epidermis thickness; ET_{ab} , abaxial epidermis thickness; MT, mesophyll thickness; SD, stomatal density; A_s , stomatal apparatus area; LMA, leaf dry mass per unit area; C_{mass} , leaf carbon concentration; N_{mass} , leaf nitrogen concentration; P_{mass} , leaf phosphorus concentration.

(61.8 \pm 11.1 mmol H₂O m⁻² s⁻¹) were significantly higher at Kunming than those at Menglun (1.62 \pm 0.04 $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$, 31.1 \pm 5.5 mmol H₂O m⁻² s⁻¹, respectively). However, except for the P_{max} being significantly higher at Kunming than at Menglun for *P. micranthum*, there was no significant difference in g_s for the two

species between Puer and Menglun. The R_d and T_r of the two species had no significant differences among the three sites, except the T_r value of *P. micranthum* was significantly higher at Puer than at Menglun. Moreover, the values of P_{max} , g_s and T_r of the two species were significantly higher in summer than in winter (Table 2).

Table 2
Differences in photosynthetic traits in *Paphiopedilum dianthum* and *P. micranthum* between summer and winter at the three study sites.

Species	Traits	Summer	Winter	P value
<i>P. dianthum</i>	R _d (μmol m ⁻² s ⁻¹)	0.60 ± 0.06	0.23 ± 0.11	0.047
	P _{max} (μmol CO ₂ m ⁻² s ⁻¹)	4.43 ± 0.01	0.71 ± 0.22	0.005
	g _s (mmol H ₂ O m ⁻² s ⁻¹)	66.0 ± 16.9	8.3 ± 2.9	0.028
	T _r (mmol H ₂ O m ⁻² s ⁻¹)	0.66 ± 0.06	0.13 ± 0.07	0.004
	WUE _i (μmol CO ₂ mmol H ₂ O ⁻¹)	7.15 ± 0.20	9.50 ± 3.93	0.611
<i>P. micranthum</i>	R _d (μmol m ⁻² s ⁻¹)	0.24 ± 0.06	0.23 ± 0.14	0.943
	P _{max} (μmol CO ₂ m ⁻² s ⁻¹)	2.24 ± 0.40	0.81 ± 0.40	0.066
	g _s (mmol H ₂ O m ⁻² s ⁻¹)	46.0 ± 8.7	14.0 ± 5.0	0.033
	T _r (mmol H ₂ O m ⁻² s ⁻¹)	0.36 ± 0.06	0.13 ± 0.02	0.021
	WUE _i (μmol CO ₂ mmol H ₂ O ⁻¹)	6.82 ± 0.51	8.46 ± 5.37	0.789

Values were means ± SE (n = 3). Analysis of difference based on t tests of independent samples and the maximum value was bold. The abbreviations are the same as Table 1.

3.2. Leaf functional traits at different sites

There were significant differences in many leaf anatomical and chemical traits for *P. dianthum* and *P. micranthum* among the three sites (Table 1). For *P. dianthum*, the values of LT, ET_{ad}, and MT were significantly higher at Puer than those at Kunming and Menglun. The SD was larger at Puer than at Menglun. The C_{mass} was significantly higher at Menglun than at Kunming and Puer. However, no significant difference was found in the CT_{ad}, CT_{ab}, ET_{ab}, A_s, LMA, N_{mass}, and P_{mass} in *P. dianthum* among the three sites. For *P. micranthum*, the LT, CT_{ad}, and MT were significantly thicker at Kunming than at Puer and Menglun. The change trend in the SD among the three sites was similar to that of *P. dianthum*. However, the values of CT_{ab}, ET_{ad}, ET_{ab}, A_s, LMA, C_{mass}, N_{mass}, and P_{mass} in *P. micranthum* was not significantly different among the three sites. The plasticity indices of LT, CT_{ab}, ET_{ad}, ET_{ab}, SD, LMA, C_{mass}, N_{mass}, and P_{mass} in *P. dianthum* among the three sites were higher than those of *P. micranthum*. Nevertheless, except for the ET_{ab} and A_s, the CT_{ad}, CT_{ab}, SD, C_{mass}, P_{mass}, R_d, P_{max} and T_r of *P. dianthum* were significantly higher than those of *P. micranthum* (Table 3).

3.3. Flowering performance at different sites

The flowering performance of *P. dianthum* was significantly different at the three sites (Table 4). The flower number was significantly fewer at Menglun than at Puer and Kunming. The scape length was significantly larger at Menglun than at Puer and Kunming, but the petal length, lip length and dorsal sepal length

Table 3
Differences in leaf anatomical and photosynthetic traits between *Paphiopedilum dianthum* and *P. micranthum* at the three study sites.

Traits	<i>P. dianthum</i>	<i>P. micranthum</i>	P value
LT (μm)	1212.8 ± 94.9	965.3 ± 40.0	0.074
CT _{ad} (μm)	33.72 ± 1.10	24.25 ± 1.55	0.008
CT _{ab} (μm)	24.60 ± 0.85	19.18 ± 0.36	0.004
ET _{ad} (μm)	422.3 ± 61.3	161.9 ± 2.9	0.051
ET _{ab} (μm)	58.19 ± 1.91	67.82 ± 0.67	0.009
MT (μm)	662.9 ± 29.4	692.6 ± 34.6	0.549
SD (mm ⁻²)	40.30 ± 2.71	17.60 ± 0.92	0.001
A _s (μm ⁻²)	1413.2 ± 57.4	2473.6 ± 150.7	0.003
LMA (g m ⁻²)	157.6 ± 5.7	169.3 ± 4.9	0.195
C _{mass} (%)	48.40 ± 0.54	43.77 ± 0.17	0.001
N _{mass} (%)	1.015 ± 0.08	0.86 ± 0.05	0.182
P _{mass} (%)	1.18 ± 0.07	0.52 ± 0.02	0.001
R _d (μmol m ⁻² s ⁻¹)	0.60 ± 0.06	0.24 ± 0.06	0.013
P _{max} (μmol CO ₂ m ⁻² s ⁻¹)	4.43 ± 0.61	2.24 ± 0.40	0.04
g _s (mmol H ₂ O m ⁻² s ⁻¹)	66.0 ± 16.9	46.0 ± 8.7	0.351
T _r (mmol H ₂ O m ⁻² s ⁻¹)	0.66 ± 0.06	0.36 ± 0.06	0.023
WUE _i (μmol CO ₂ mmol H ₂ O ⁻¹)	7.15 ± 0.20	6.82 ± 0.51	0.578

Values were means ± SE (n = 3). Analysis of difference based on t tests of independent samples and the maximum value was bold. The abbreviations are the same as Table 1.

showed significant opposite trends. However, the floral buds of *P. micranthum* were aborted before blossoming at Menglun, while the plants at Puer and Kunming flowered.

4. Discussion

We found in this study that the photosynthetic rate, leaf thickness and stomatal density of the two *Paphiopedilum* species showed the same changing trend in response to temperature. Warming significantly reduced photosynthetic rate, leaf thickness and stomatal density, and inhibited the flower performances of the two *Paphiopedilum* species. Compared with *P. dianthum*, *P. micranthum* showed less resistance to elevated temperature in flower performances.

In this study, the photosynthetic traits of *P. dianthum* and *P. micranthum* showed significant differences under various environmental temperatures. Among the three study sites, the P_{max} of the two *Paphiopedilum* species was the lowest at Menglun, where the annual average temperature and air relative humidity were the highest (Fig. 2, Table 1). Compared with that at Kunming, the P_{max} of *P. dianthum* was decreased by 38% at Menglun, while the P_{max} of *P. micranthum* was decreased by 46%. Contrary to our result, an increased temperature increases the P_{max} of *Dryas Octopetala* var. *asiatica* and *Rhododendron confertissimum* (Zhou et al., 2019). Such inconsistency may be caused by differences in the optimum temperature requirement of different species. Both *P. dianthum* and *P. micranthum* are distributed in limestone areas above 1000 m a.s.l. in subtropical Asia (Cribb, 1998), where the temperature is not too high. However, Menglun (alt. 570 m) locates at the northern edge of the tropics has high air relative humidity and temperature. The summer temperature at Menglun may exceed the annual average temperature (about 16 °C) of natural distribution area of the two *Paphiopedilum* species. Thus, the P_{max} at Menglun may be depressed by high temperatures. Previous studies have suggested that the g_s and Rubisco activity of plants decrease under high temperature conditions (Greer and Weston, 2010; Greer and Weedon, 2011; Wise et al., 2004). Compared with *P. dianthum*, the P_{max} of *P. micranthum*

Table 4
Difference in flowering performance of *Paphiopedilum dianthum* at the three study sites.

Traits	Study sites		
	Menglun	Puer	Kunming
Number of flowers per scape	1.5 ± 0.0 ^b	2.7 ± 0.1^a	2.3 ± 0.1 ^a
Scape length (cm)	35.02 ± 0.96^a	27.89 ± 0.90 ^b	28.09 ± 0.86 ^b
Petal length (cm)	9.21 ± 0.39 ^b	11.69 ± 0.17 ^a	11.80 ± 0.16^a
Lip length (cm)	4.54 ± 0.10 ^b	4.84 ± 0.04^a	4.83 ± 0.04 ^a
Dorsal sepal length (cm)	4.53 ± 0.13 ^b	5.11 ± 0.07 ^a	5.29 ± 0.05^a

Values were means ± SE (n = 5). Different letters indicated significant differences between study sites and the maximum value was bold (P < 0.05, based on ANOVA, followed by Tukey's tests for comparison).

at Menglun was reduced by a greater proportion. These results showed that the two *Paphiopedilum* species had weak tolerance to high temperature at Menglun, and *P. micranthum* was less tolerant to high temperature than *P. dianthum*. The reason why *P. micranthum* was less tolerant to high temperature than *P. dianthum* was that the former was more geographically distributed in the North (Fig. 1). In addition, the photosynthetic rates of the two *Paphiopedilum* species in winter were significantly lower than that in summer. This might be related to the seasonal variation in the vigor of *Paphiopedilum* plants which was affected by seasonal climate. Thus, growth temperature was a key factor affecting the photosynthetic performances of two *Paphiopedilum* species.

The changes in leaf anatomical traits at the three sites were species-specific. For *P. dianthum*, the values of LT, ET_{ad} , MT, and SD were significantly higher at Puer than those at Menglun, but were not significantly different between Puer and Kunming. This indicated that leaf anatomical traits of *P. dianthum* were significantly affected by temperature. Previous studies have suggested that LT is significantly reduced under warming environments (Jin et al., 2011; Yang et al., 2011; Zheng et al., 2013; Habermann et al., 2019; Zhou et al., 2019). In this study, the change of LT of *P. dianthum* was 23.9% which was mainly due to the change of ET_{ad} and MT. Thus, high temperature might decrease LT, mainly via reducing the ET_{ad} and MT of *P. dianthum*. A high temperature is usually accompanied by high water loss. To reduce water loss via stomata, plants usually regulate the number or area of stomata (Xu and Zhou, 2008; Cruz et al., 2019). Here, the SD in two *Paphiopedilum* species reduced significantly under warmer conditions. This is consistent with the result from a study on *S. superba* (Wu et al., 2018). Thus, the low photosynthetic rate in *P. dianthum* at Menglun was related to the reduced SD. For *P. micranthum*, the values of LT, CT_{ad} , and MT were significantly higher at Kunming than those at Menglun. This is consistent with previous results (Bacelar et al., 2004; Cassola et al., 2019). The increase in cuticle thickness is a response of plant development to water deficits (Chen et al., 2020). Plants may increase the CT_{ad} to prevent water loss under conditions of high transpiration demand (Bacelar et al., 2004). There were no significant differences in the values of CT_{ab} , ET_{ad} , ET_{ab} , and A_s among the three sites. The LT and SD of the two *Paphiopedilum* species are important traits in response to the changing growth temperatures. Thus, the adjustments of LT and SD of *Paphiopedilum* species may play essential roles in plant responses to different environmental temperatures. In addition, some leaf anatomical traits of *P. dianthum* were significantly higher than those of *P. micranthum*, such as the thickness of cuticles and stomatal densities. Thus, these variations in leaf anatomical traits may help *P. dianthum* to adapt to environmental change better than *P. micranthum*.

The value of C_{mass} in the two species was higher at Menglun than at Kunming, but there were no significant differences in N_{mass} , P_{mass} , and LMA among the three sites. Compared with *P. dianthum*, the values of N_{mass} and P_{mass} were higher than those in *P. micranthum*, while the value of LMA was opposite in the two species. Previous studies have also suggested that the plants with higher LMA have lower values of N_{mass} and P_{mass} (Guan et al., 2011), and LMA shows a significant increase in drought environments (Wang et al., 2011; Toscano et al., 2018). This indicated that the two *Paphiopedilum* species were not stressed by water availability in summer. However, the two species become dormant during winter, and no new leaves are produced. Thus, further research is still required to illustrate the response of *Paphiopedilum* species to water status.

The P_{max} is influenced by leaf anatomical and physiological traits. Leaf photosynthesis depends on the concentration of CO_2 which arrives at the carboxylation site (Muir et al., 2014). Stomata, intercellular airspaces, cells, plasma membrane, cytosol, and chloroplast envelopes and stroma are the major barriers that limit atmospheric

CO_2 arrival at the carboxylation site (Flexas et al., 2012; Muir et al., 2014). A previous study has found that there is a significantly positive correlation between photosynthetic rate and g_s (Gago et al., 2016). The g_s is determined by SD, stomatal size and whether the stomata are open (Franks and Beerling, 2009; De Boer et al., 2016). In our study, the change trend of P_{max} was same with g_s , SD, LT, CT_{ab} , ET_{ad} , but opposite with ET_{ab} and A_s among the three sites (Table 1). This is similar to previous studies which found that the P_{max} increases with SD (Xu and Zhou, 2008; Jin et al., 2011; Yang et al., 2018). There was no significant difference in the A_s among the three sites, which further proved that the two species may not be stressed by drought (Xu and Zhou, 2008). In addition, the increase in ET_{ab} may decrease the mesophyll conductance, which is positively correlated with the photosynthetic rate (Grassi and Magnani, 2005). Thus, leaf anatomy plays an important role in regulating photosynthesis of two *Paphiopedilum* species in different growth environments.

Temperature has a vital influence on flowering traits, the flowering of *Doritaenopsis* 'Newberry Parfait' was completely suppressed under high-temperature (Newton and Runkle, 2009). However, high temperature can increase the inflorescence size of *Phalaenopsis* hybrid (Lee and Lin, 1984), and leads to the reduction of flower number and size of tepals in *Phalaenopsis* hybrid and *Tipularia discolor* (Newton and Runkle, 2009; Marchin et al., 2014). In our study, the flowering performance of the two *Paphiopedilum* species were significantly different at the three sites. The number of flowers of *P. dianthum* was significantly fewer, and no normal flowers developed on the *P. micranthum* plants at Menglun. High temperature significantly decreased the length of petal, dorsal sepal, and lip of *P. dianthum* (Table 4). We inferred that there was a trade-off between inflorescence size and tepals size of *Paphiopedilum* species under high temperature conditions. These results not only indicated that high temperature inhibited the flowering performances of the two *Paphiopedilum* species, but also revealed that *P. dianthum* might be tolerant to high temperature better than *P. micranthum*.

Plant plasticity has been recognized as a vital aspect of how plants develop, function and evolve in their environments (Sultan, 2000). High phenotypic plasticity is not only associated with wide geographical distribution of a species, but it also demonstrates the ability of plants to adapt to new environments (Sultan, 2000; Velikova et al., 2020). Moreover, greater phenotypic plasticity would enable a plant to respond more rapidly to an adverse environment in a short time, which shows that these species may accelerate the process of adaptation in new environments (Ghalambor et al., 2007; McLean et al., 2014). In our study, compared with *P. micranthum*, higher plasticity indexes of *P. dianthum* were found in LT, CT_{ad} , CT_{ab} , ET_{ad} , ET_{ab} , SD, LMA, C_{mass} , N_{mass} , and P_{mass} . In particular, the plasticity indices of LT, ET_{ab} , and SD of *P. dianthum* were larger than those of *P. micranthum*. Similar to our hypothesis, these results indicated that the stronger adaptability of *P. dianthum* than *P. micranthum* was related to the plasticity of leaf traits under the tested environments.

In conclusion, we focused on the leaf photosynthetic, anatomical, and flowering responses of *P. dianthum* and *P. micranthum* to different environmental temperatures in southwest China. The photosynthetic rate and flowering performance of the two species were strongly affected by temperature. However, compared with *P. micranthum*, the photosynthetic rate of *P. dianthum* was less sensitive to high temperature. The leaf thickness, mesophyll thickness, and stomatal density were lower at Menglun than those at Puer and Kunming. Furthermore, a larger plasticity of leaf anatomical traits makes *P. dianthum* more adaptable to the tested environments. This study indicates that *P. dianthum* plants can more effectively regulate their leaf structure to respond to various environmental temperatures than *P. micranthum*. The findings will contribute to the conservation and utilization of *Paphiopedilum* species.

Author contributions

S.B.Z. and J.Q.F. designed the study; J.Q.F. carried out the experiments; J.Q.F. and S.B.Z. analyzed the data; J.Q.F., J.H.W. and S.B.Z. wrote and revised this manuscript.

Declaration of competing interest

The authors declare no competing financial interest.

Acknowledgements

This work was financially supported by the National Natural Science Foundation of China (31970361), the Applied Basic Research Plan of Yunnan Province (2018FA016), the Science and Technology Plan of Yunnan (2018BB010), and the project for Construction of International Flower Technology Innovation Center and Achievement Industrialization (2019ZG006), and the Project for Innovation Team of Yunnan Province. Thanks to Dr. John A Meadows for proofreading and editing. Thanks to Mr. Jianbo Yang for helping to make the map of species distribution.

References

- An, H.R., Kim, Y.J., Kwon, O.K., et al., 2017. High temperature promotes growth and flowering in *Sophrolaeliocattleya*. *Hortic. Environ. Biotechnol.* 58, 268–273.
- Assmann, S.M., Zeiger, E., 1985. Stomatal responses to CO₂ in *Paphiopedilum* and *Phragmipedium*—role of the guard cell chloroplast. *Plant Physiol.* 77, 461–464.
- Averyanov, L., Cribb, P., Ke Loc, P., et al., 2003. Slipper Orchids of Vietnam. Royal Botanical Gardens, Kew.
- Bacelar, E.A., Correia, C.M., Pereira, J.M.M., et al., 2004. Sclerophylly and leaf anatomical traits of five field-grown olive cultivars growing under drought conditions. *Tree Physiol.* 24, 233–239.
- Bleho, B.I., Borkowsky, C.L., Grantham, M.A., et al., 2021. A 20 y analysis of weather and management effects on a small white lady's-slipper (*Cypripedium candidum*) population in Manitoba, Am. Midl. Nat. 185, 32–48.
- Cassola, F., Silva, M.H.R., Borghi, A.A., et al., 2019. Morphoanatomical characteristics, chemical profiles, and antioxidant activity of three species of *Justicia* L. (Acanthaceae) under different growth conditions. *Ind. Crop. Prod.* 131, 257–265.
- Chen, M.J., Zhu, X.F., Zhang, Y., et al., 2020. Drought stress modify cuticle of tender tea leaf and mature leaf for transpiration barrier enhancement through common and distinct modes. *Sci. Rep.* 10, 6696.
- CITES, 2012. Convention on international trade in endangered species of wild fauna and flora. Appendices I, II and III. <http://www.cites.org>.
- Cribb, P., 1998. The Genus *Paphiopedilum*, second ed. Natural History Publications, Kew, UK. Kota Kinabalu (Borneo) in association with Royal Botanic Gardens.
- Cruz, Y.D.C., Scarpa, A.L.M., Pereira, M.P., et al., 2019. Growth of *Typha domingensis* as related to leaf physiological and anatomical modifications under drought conditions. *Acta Physiol. Plant.* 41, 64.
- De Boer, H.J., Price, C.A., Wagner-Cremer, F., et al., 2016. Optimal allocation of leaf epidermal area for gas exchange. *New Phytol.* 210, 1219–1228.
- Ferris, R., Nijs, I., Behaeghe, T., et al., 1996. Elevated CO₂ and temperature have different effects on leaf anatomy of perennial ryegrass in spring and summer. *Ann. Bot.* 78, 489–497.
- Flexas, J., Barbour, M.M., Brendel, O., et al., 2012. Mesophyll diffusion conductance to CO₂: an unappreciated central player in photosynthesis. *Plant Sci.* 193, 70–84.
- Franks, P.J., Beerling, D.J., 2009. Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time. *Proc. Natl. Acad. Sci. U. S. A.* 106, 10343–10347.
- Gago, J., Daloso, D.M., Figueroa, C.M., et al., 2016. Relationships of leaf net photosynthesis, stomatal conductance, and mesophyll conductance to primary metabolism: a multispecies meta-analysis approach. *Plant Physiol.* 171, 265–279.
- Ghalambor, C.K., McKay, J.K., Carroll, S.P., et al., 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. *Funct. Ecol.* 21, 394–407.
- Grassi, G., Magnani, F., 2005. Stomatal, mesophyll conductance and biochemical limitations to photosynthesis as affected by drought and leaf ontogeny in ash and oak trees. *Plant Cell Environ.* 28, 834–849.
- Greer, D.H., Weston, C., 2010. Heat stress affects flowering, berry growth, sugar accumulation and photosynthesis of *Vitis vinifera* cv. Semillon grapevines grown in a controlled environment. *Funct. Plant Biol.* 37, 206–214.
- Greer, D.H., Weedon, M.M., 2011. Modelling photosynthetic responses to temperature of grapevine (*Vitis vinifera* cv. Semillon) leaves on vines grown in a hot climate. *Plant Cell Environ.* 35, 1050–1064.
- Guan, Z.J., Zhang, S.B., Guan, K.Y., et al., 2011. Leaf anatomical structures of *Paphiopedilum* and *Cypripedium* and their adaptive significance. *J. Plant Res.* 124, 289–298.
- Habermann, E., San Martin, J.A.B., Contin, D.R., et al., 2019. Increasing atmospheric CO₂ and canopy temperature induces anatomical and physiological changes in leaves of the C₄ forage species *Panicum maximum*. *PLoS One* 14, e0212506.
- Iersel, M.W.V., 2003. Short-term temperature change affects the carbon exchange characteristics and growth of four bedding plant species. *J. Am. Soc. Hortic. Sci.* 128, 100–106.
- Jin, B., Wang, L., Wang, J., et al., 2011. The effect of experimental warming on leaf functional traits, leaf structure and leaf biochemistry in *Arabidopsis thaliana*. *BMC Plant Biol.* 11, 35.
- Lee, N., Lin, G.M., 1984. Effect of temperature on growth and flowering of *Phalaenopsis* white hybrid. *J. Chin. Soc. Hortic. Sci.* 30, 223–231.
- Liu, Z.J., Chen, S.C., Chen, L.J., et al., 2009. The Genus *Paphiopedilum* in China. Science Press, Beijing, pp. 4–12.
- Long, S.P., Zhu, X.G., Naidu, S.L., et al., 2006. Can improvement in photosynthesis increase crop yields? *Plant Cell Environ.* 29, 315–330.
- Marchin, R.M., Dunn, R.R., Hoffmann, W.A., 2014. Are winter-active species vulnerable to climate warming? A case study with the wintergreen terrestrial orchid, *Tipularia discolor*. *Oecologia* 176, 1161–1172.
- Matesanz, S., Gianoli, E., Valladares, F., 2010. Global change and the evolution of phenotypic plasticity in plants. *Ann. N. Y. Acad. Sci.* 1206, 35–55.
- McLean, E.H., Prober, S.M., Stock, W.D., et al., 2014. Plasticity of functional traits varies clinally along a rainfall gradient in *Eucalyptus tricarpa*. *Plant Cell Environ.* 37, 1440–1451.
- Muir, C.D., Hangarter, R.P., Moyle, L.C., et al., 2014. Morphological and anatomical determinants of mesophyll conductance in wild relatives of tomato (*Solanum* sect. *Lycopersicon*, sect. *Lycopersicoideae*; Solanaceae). *Plant Cell Environ.* 37, 1415–1426.
- Newton, L.A., Runkle, E.S., 2009. High-temperature inhibition of flowering of *Phalaenopsis* and *Doritaenopsis* orchid. *Hortscience* 44, 1271–1276.
- Pollet, B., Steppe, K., Vanlabeke, M.C., et al., 2009. Diurnal cycle of chlorophyll fluorescence in *Phalaenopsis*. *Photosynthetica* 7, 309–312.
- Poulos, H.M., Goodale, U.M., Berlyn, G.P., 2007. Drought response of two Mexican oak species, *Quercus laceyi* and *Q. sideroxylla* (Fagaceae), in relation to elevation position. *Am. J. Bot.* 94, 809–818.
- Richardson, A.D., Ashton, P.M.S., Berlyn, G.P., et al., 2001. Within-crown foliar plasticity of western hemlock, *Tsuga heterophylla*, in relation to stand age. *Ann. Bot.* 88, 1007–1015.
- Richards, C.L., Bossdorf, O., Muth, N.Z., et al., 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecol. Lett.* 9, 981–993.
- Sekiya, N., Yano, K., 2008. Stomatal density of cowpea correlates with carbon isotope discrimination in different phosphorus, water and CO₂ environments. *New Phytol.* 179, 799–807.
- Soudzilovskaia, N.A., Elumeeva, T.G., Onipchenko, V.G., et al., 2013. Functional traits predict relationship between plant abundance dynamic and long-term climate warming. *Proc. Natl. Acad. Sci. U. S. A.* 110, 18180–18184.
- Sultan, S.E., 2000. Phenotypic plasticity for plant development, function and life history. *Trends Plant Sci.* 5, 537–542.
- Toscano, S., Ferrante, A., Tribulato, A., et al., 2018. Leaf physiological and anatomical responses of *Lantana* and *Ligustrum* species under different water availability. *Plant Physiol. Biochem.* 127, 380–392.
- Valladares, F., Martinez-Ferri, E., Balaguer, L., et al., 2000. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy? *New Phytol.* 148, 79–91.
- Velikova, V., Arena, C., Izzo, L.G., et al., 2020. Functional and structural leaf plasticity determine photosynthetic performances during drought stress and recovery in two *Platanus orientalis* populations from contrasting habitats. *Int. J. Mol. Sci.* 21, 3912.
- Wang, R.Z., Huang, W.W., Chen, L., et al., 2011. Anatomical and physiological plasticity in *Leymus chinensis* (Poaceae) along large-scale longitudinal gradient in northeast China. *PLoS One* 6, e26209.
- Wise, R.R., Olson, A.J., Schrader, S.M., et al., 2004. Electron transport is the functional limitation of photosynthesis in field-grown Pima cotton plants at high temperature. *Plant Cell Environ.* 27, 717–724.
- Wu, G.L., Liu, H., Hua, L., et al., 2018. Differential responses of stomata and photosynthesis to elevated temperature in two co-occurring subtropical forest tree species. *Front. Plant Sci.* 9, 467.
- Wu, G.Y., Hui, J.A., Wang, Z.H., et al., 2014. Photosynthetic characteristics of four wild *Dendrobium* species in China. *Hortscience* 49, 1023–1027.
- Xu, Z.Z., Zhou, G.S., 2008. Response of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.* 59, 3317–3325.
- Yang, Y., Wang, G.X., Klanderud, K., et al., 2011. Responses in leaf functional traits and resource allocation of a dominant alpine sedge (*Kobresia pygmaea*) to climate warming in the Qinghai-Tibetan Plateau permafrost region. *Plant Soil* 349, 377–387.
- Yang, Y.J., Chang, W., Huang, W., et al., 2017. The effects of chilling-light stress on photosystem I and II in three *Paphiopedilum* species. *Bot. Stud.* 58, 53.
- Yang, Z.H., Huang, W., Yang, Q.Y., et al., 2018. Anatomical and diffusional determinants inside leaves explain the difference in photosynthetic capacity between *Cypripedium* and *Paphiopedilum*, Orchidaceae. *Photosynth. Res.* 136, 315–328.

- Zhang, F.P., Yang, Y.J., Yang, Q.Y., et al., 2017. Floral mass per area and water maintenance traits are correlated with floral longevity in *Paphiopedilum* (Orchidaceae). *Front. Plant Sci.* 8, 501.
- Zhang, S.B., Guan, Z.J., Chang, W., et al., 2011. Slow photosynthetic induction and low photosynthesis in *Paphiopedilum armeniacum* are related to its lack of guard cell chloroplast and peculiar stomatal anatomy. *Physiol. Plantarum* 142, 118–127.
- Zhang, S.B., Guan, Z.J., Sun, M., et al., 2012. Evolutionary association of stomatal traits with leaf vein density in *Paphiopedilum*, Orchidaceae. *PLoS One* 7, e40080.
- Zheng, Y.P., Xu, M., Shen, R.C., et al., 2013. Effects of artificial warming on the structural, physiological, and biochemical changes of maize (*Zea mays* L.) leaves in northern China. *Acta Physiol. Plant.* 35, 2891–2904.
- Zhou, Y.M., Deng, J.F., Tai, Z.J., et al., 2019. Leaf anatomy, morphology and photosynthesis of three tundra shrubs after 7-year experimental warming on Changbai Mountain. *Plants* 8, 271.