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Diurnal changes in gas exchange and chlorophyll fluorescence parameters of *Fritillaria cirrhosa* and *F. delavayi* under field conditions

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Abstract

To determine what factors limit the growth of wild *Fritillaria cirrhosa* and *Fritillaria delavayi* in field conditions, we investigated diurnal changes of the net photosynthetic rate (P_N) and the correlation between P_N and various environmental factors. Parameters of chlorophyll (Chl) fluorescence were evaluated to test whether ecological fragility caused the extinction of wild *F. cirrhosa* and *F. delavayi.* Our study reveals for the first time that *F. cirrhosa* and *F. delavayi* did not encounter significant stress under field conditions. A small reduction in maximum photochemical efficiency was observed under high irradiance. The maximum P_N of *F. cirrhosa* was 30 % higher than *F. delavayi* ($p<0.05$), and a similar difference was observed for apparent quantum yield (27.3 %, $p<0.01$). *F. delavayi* was better adapted to a wide range of irradiances and high environmental temperature. Correlation between P_N and environmental factors (without considering the effects of interactions among environmental factors on P_N) using leaves of *F. cirrhosa* revealed that the three primary influencing factors were air pressure ($p<0.01$), relative humidity ($p<0.01$), and soil temperature ($p<0.05$). In *F. delavayi*, the influencing factors were relative humidity ($p<0.01$), soil temperature ($p<0.05$), CO_2 concentration ($p<0.05$), and air pressure ($p<0.05$). Path analysis (considering effects among environmental factors on P_N) showed that air temperature (negative correlation), photosynthetic photon flux density (PPFD) and relative humidity were the three primary limiting factors influencing the growth of *F. cirrhosa*. For this species, relative humidity reacted indirectly with air pressure, which was reported singularly in other species. Limiting growth factors for *F. delavayi* were PPFD, air pressure (negative correlation), soil temperature (negative correlation) and air temperature (negative correlation).

Additional key words: chlorophyll fluorescence; *F. cirrhosa*; *F. delavayi;* path analysis; photosynthesis.

Introduction

F. cirrhosa and *F. delavayi* are two endemic species in the Chuanxi altiplano of China that have become threatened in the wild. The bulbs of these two species are used as medicinal materials. They are third-grade protected species in China and accepted by Chinese Pharmacopoeia (2005) and are regarded as the two best traditional Chinese medicines for relieving cough and eliminating phlegm. The manufacture of *Fritillaria* preparations has developed into a large-scale industry worth an estimated US$ 400 million a year in China, and there are currently 400 manufacturers producing over 200 kinds of *Fritillaria* preparations. Even so, the demand for *Fritillaria* products is growing quickly, and the annual output covers only 5 % of the market demand (UNDP 2005). Unfortunately, large-scale field cultivation of these species has not been successful. Re-introducing the species into their natural habitats yields low production, which leads to the collection of wild individuals at a rate exceeding the natural supply. These factors have pushed wild *F. cirrhosa* and *F. delavayi* to the brink of extinction. There has been extensive documentation of these two species' chemistry (Wang *et al.* 2003a),

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Abbreviations: AQY – apparent quantum yield; C_i – intercellular CO_2 concentration; Chl – chlorophyll; E – transpiration rate; ETR – electron transport rate; F_0 – initial, F_v – variable, and F_m – maximum chlorophyll fluorescence, respectively; g_s – stomatal conductance; LCP – light compensated point; LSP – light saturated point; NPQ – non–photochemical quenching; P_N – net photosynthetic rate; PPFD – photosynthetic photon flux density; q_P – photochemical quenching; R_D – dark respiration; T_{air} – air temperature; T_{soil} – soil temperature; VPD – vapour pressure deficit; WUE – water use efficiency; Φ_{PSII} – quantum yield of PSII.

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pharmacology (Li *et al.* 1995), molecular biology (Lin *et al.* 2006), and community (Chen *et al.* 2003). Despite their high economic value, there are very few gas exchange and ecological adaptability studies on these two species.

F. cirrhosa lives at altitudes from 3200 m to 4500 m and *F. delavayi* grows at altitudes from 3800 m to 4700 m. They have similar kinds of alkaloids which mainly derived from stresses (Huang *et al.* 1997). We hypothesized that the two species may have similar limiting environmental factors on gas exchange according to the perspective of "daodi" theory (Huang *et al.* 1997). In this study, we investigated diurnal changes of net photosynthetic rate (P_N) and relative parameters, in addition to correlations between P_N and environmental factors by path analysis. This was done to help determine which factors limit gas exchange under field conditions for the two species. Parameters of Chl fluorescence were evaluated to test whether ecological fragility was the major cause of extinction and failure in cultivation of wild *F. cirrhosa* and *F. delavayi.* It was the final objective to provide a basic information for cultivation.

Materials and methods

Plants and growing conditions: The experiment was conducted at a research field station located in MaiBa village, Kangding County, China (E30º03′49″ , N101º34′58″, 3535 m a. s. l.). *F. cirrhosa* and *F. delavayi* were introduced from mountains nearby (Fig. 1.). This area belongs to the frigid and variable zone, with large seasonal temperature variations (from –20 to +25 °C). The main characteristics of the climate are dry, windy spring; cool, rainy summer with frequent hailstorms; dry, short autumn with early frosts; and long, cold winter with much snow. There is a high photosynthetic photon flux density (PPFD) during the day (maximum extreme values are from 2800 to 3000 μmol m^{-2} s^{-1}) and a high evapotranspiration. The annual precipitation ranges from 800 to 900 mm, falling mainly from June to August.

Bulbs of the two species were collected in September 2005 after the aboveground plants wilted and were planted on October 8, 2005 under identical conditions at the field research station. The original habitat and community environments of wild *F. cirrhosa* were that: average PPFD was from 1000 to 1400 μmol m^{-2} s^{-1}; average content of soil water was beyond 60 %; air temperature (T_{air}) was lower than 25 ºC, air relative humidity was higher than 65 %.

Gas exchange and related parameters: *F. cirrhosa* and *F. delavayi* spend a whole lifecycle of about 50 days with expanded green leaves in a year. We measured the maximum photosynthetic rate in flowering stage which was their blooming phase. The flowering stage takes about 30 days.When flowers of *F. cirrhosa* and *F. delavayi* were full-blown, gas exchange parameters were investigated from June 5 to July 7, in 2006 under natural environmental conditions**.** The parameters of gas exchange were measured on middle, fully expanded leaves of plants between the hours of 06:00 and 18:00 during bright sunlight on a clear, cloudless day (average T_{air} was 27 °C, average PPFD was beyond 1800 μmol m^{-2} s^{-1}, and average air relative humidity was 40 %, content of soil water was lower than 30 %) including P_N, PPFD, transpiration rate (E), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), dark respiration (R_D), air pressure, T_{air}, relative humidity, air CO_2 concentration *etc*., using a portable photosynthesis system (*LI-6400*-sun-sky, *LI-COR,* Lincoln, USA). Soil temperature (T_{soil}) was measured with geothermometer *(RM-003, Ruiming Company*, Changzhou, China). Vapour pressure deficit (VPD) was estimated by equation VPD = $e(T_l)–e_s$ e(T), where e(T) is the saturation vapor pressure function (Buck 1981), T_l the leaf temperature, and e_s is vapor pressure in the sample cell. Parameters were recorded once per hour. Two leaves were analysed for each plant at each time, and three replications were performed.

Light response curves (PPFD-P_N) were obtained using a portable photosynthesis system (*LI-6400-02*, red-blue, *LI-COR*, Lincoln, USA) with a controlled atmosphere [approximately 390 μmol (CO_2) m^{-2} s^{-1}, relative humidity from 40 to 60 %, 0.5 L/min air flow and 20 ± 2 ºC]. The PPFD was designed as follows: 2000, 1800, 1600, 1400, 1200, 1000, 800, 600, 400, 200, 150, 100, 50, 20, and 0 μmol m^{-2} s^{-1}. The parameters of maximum photosynthetic rate (P_{max}), E, R_D, apparent quantum yield (AQY), light saturated point (LSP), light compensated point (LCP), and water use efficiency (WUE) were measured. WUE was estimated by P_N/E.

Chl *a* fluorescence parameters were monitored under field conditions using a portable pulse amplitude modulation fluorometer (*LI-6400-40, LI-COR*, Lincoln, USA). Minimal and maximal fluorescence of a dark-adapted leaf, F_0 and F_m, respectively, were measured before dawn (2:00～4:00) and in the afternoon (13:00～15:00) after a 40 min dark adaptation to calculate maximum photochemical efficiency (F_v/F_m). PPFD was controlled coincident with natural light. Natural PPFD is higher than 2000 μmol m^{-2} s^{-1} and T_{air} is beyond 30 ºC. Efficiency of energy harvesting in the light (F_v'/F_m'), values of the effective quantum yield of PSII (Φ_{PSII}) photochemistry, photochemical quenching (q_P), non-photochemical quenching (NPQ), and electron transport rate values (ETR) were measured. ETR was calculated by the equation ETR = $(F_m'–F_s)$ f I α_{leaf}/F_m' where F_m' is the maximal fluorescence during a saturating light flash and F_s is steady-state fluorescence, f = 0.5, I is an incident photo flux density, and α_{leaf} is a leaf absorptance). Detailed operation and setting referred to the guide of *LI-6400-40* portable photosynthesis system. The results shown are means ± SD of the measurements made on 10 different leaves (one leaf per plant).

Data analysis: P_N was calculated using the regression model ($P_N = P_{max}$ (1–C_0 $e^{-\alpha PPFD/P_{max}}$) where C_0 is a constant, $C_0\alpha$ is a quantum efficiency of CO_2 assimilation in weak light. LCP was estimated using the model (LCP = P_{max} 1n(C_0)/α), and LSP was calculated by (LSP = P_{max} 1n(100 C_0)/α). The correlation between P_N and environmental factors (Pearson correlation coefficient) was evaluated using *Statistical Package for Social Sciences* software (*SPSS*, 13.0). Path analysis was performed using *Data Processing System* software (Tang 2002). Path analysis model was drawn (Fig 1) according to path models in Ball (2001) and Das (2004). Eight variables were included in the path analysis: Y (P_N), X_1 (T_{air}), X_2 (CO_2 concentration), X_3 (relative humidity), X_4 (PPFD), X_5 (T_{soil}), X_6 (soil humidity), and X_7 (air pressure). Each variable was measured 13 times in a curve of diurnal variation. Six replications were performed. All data were imported into *DPS* software and underwent path analysis to detect path coefficients.

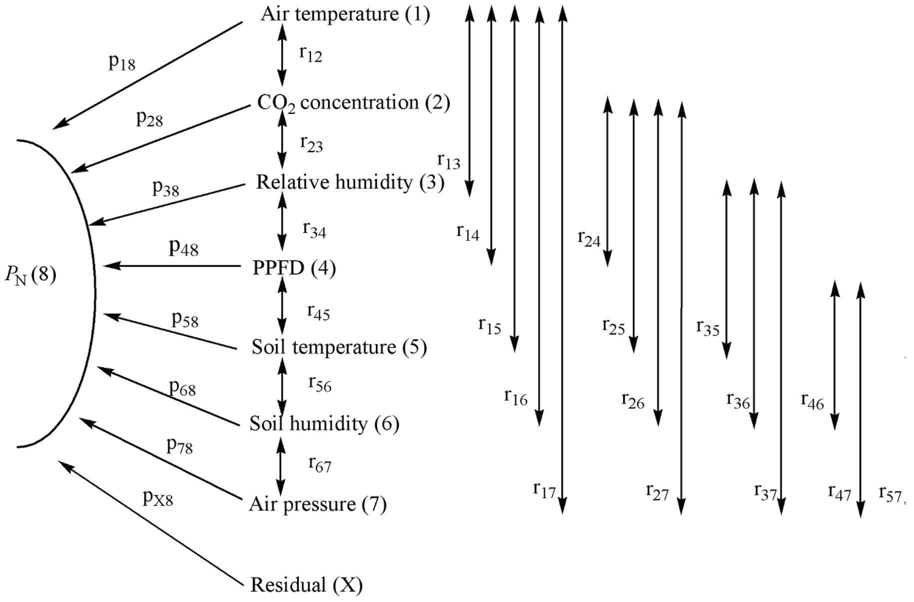

Fig. 1. Path model of the effects of environmental factors on P_N. Unidirectional arrows (→) represent direct path (p) and bidirectional arrows (↔) represent correlations (r).

Results and discussion

Diurnal changes of photosynthetic parameters: Diurnal changes of leaf P_N showed similar circadian rhythms in the two species (Fig. 2*A*). P_N of *F. cirrhosa* increased very quickly early in the morning and then decreased sharply, reaching a minimum at about 14:00, followed by a slow recovery in the later afternoon, which can be considered typical behaviour in altiplano plants. Although *F. delavayi* had two peaks, the second was not clear. The values of P_N from 10:00 to 17:00 remained in a relative steady state, and another difference was that P_N showed a slight increase to 5.68 μmol $m^{-2}s^{-1}$ at 14:00 when PPFD reached beyond 2100 μmol m^{-2} s^{-1}, indicating that *F. delavayi* was more adaptive to irradiance and high temperature stress. This result coincides with the two species' natural habitats. Because both of their minimal P_N values were above 0 μmol m^{-2} s^{-1}, the two species appear to have adapted well to so high a PPFD (Lu *et al.* 1995). Therefore, high irradiance may not be the main cause of the extinction of the two species. PPFD affected plant growth and morphological features. The leaves of *F. cirrhosa* are strip-shaped and thin, of a shade-tolerant character. *F. delavayi* leaves are elliptical, thicker and succulent, reflecting drought tolerance and allowing the plant to adapt to its growing conditions at the research station (high irradiance and drought) more easily than *F. cirrhosa*.

Atmospheric evaporative demand and, consequently, crop transpiration increase with increasing atmospheric vapour pressure deficit (VPD) (Sinclair and Bennett 1998). However, *F. cirrhosa* showed a reverse result (Fig. 2*B*). Sinclair *et al.* (2005) demonstrated that the imposition of limited maximum transpiration rates increased sorghum yields in 76–90 % of seasons in a semi-arid environment. This outcome was due both to the water savings associated with reduced transpiration and to increased WUE. Although *F. delavayi* had higher leaf water content (LWC) (86.73 %, unpublished data) and a lower VPD, WUE was lower (Fig. 2*F*), which may be caused by high *E*. This decreased P_N verifies the report by Sinclair. A limited maximum *E* that is reached when atmospheric VPD exceeds 2.0 kPa would result in an afternoon depression of photosynthesis (Hirasawa and

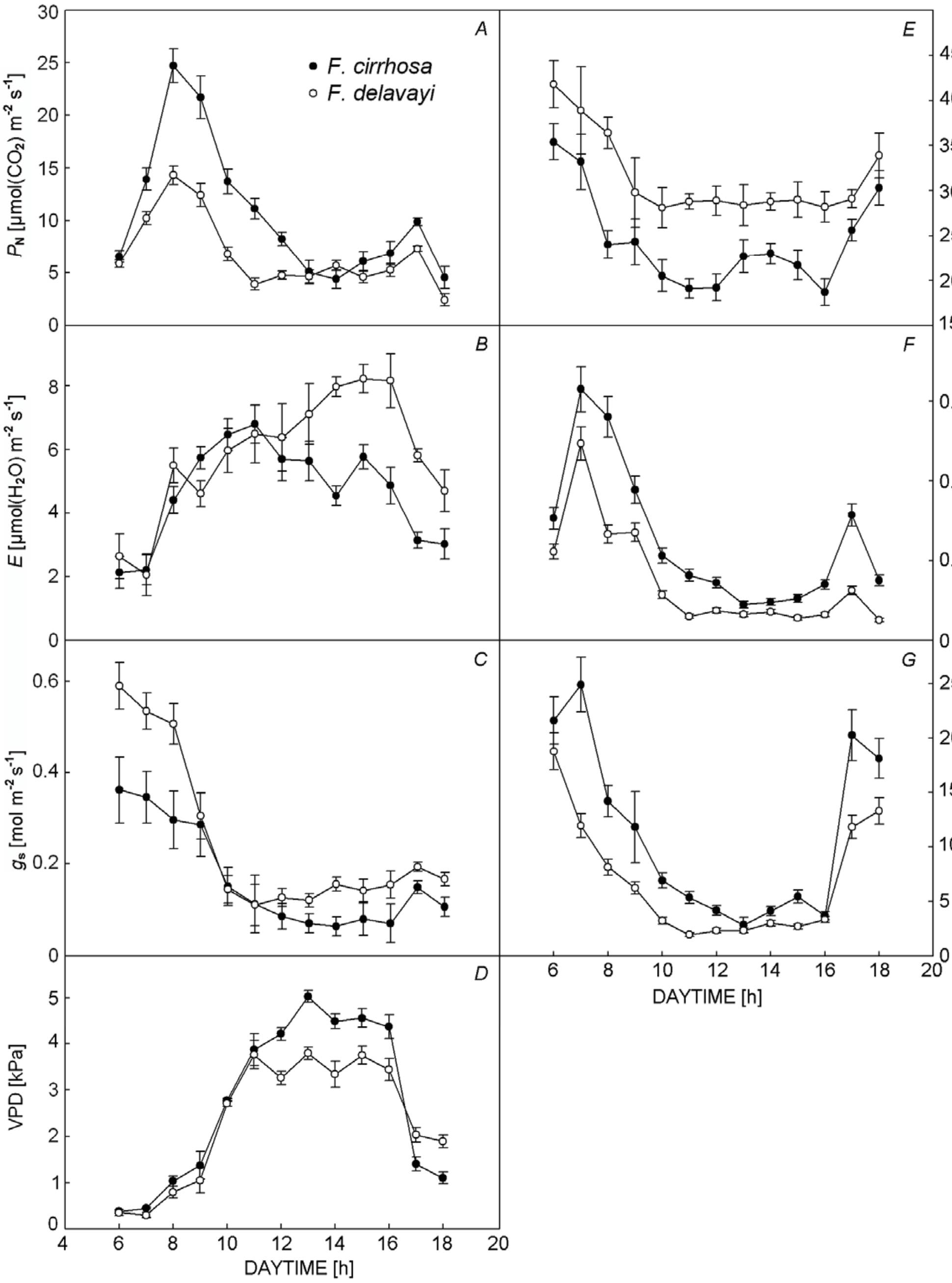

Fig. 2. Curves of diurnal variation of *A*: net photosynthetic rate, P_N; *B*: transpiration rate, *E*; *C*: stomata conductance, g_s; *D*: vapour pressure deficit, VPD; *E*: intercellular CO_2 concentration, C_i; *F*: water use efficiency, WUE; *G*: apparent quantum yield, AQY. Each value represents the means ± SE (*n*=6).

Hsiao 1999). The correlation between VPD and P_N in the two fritillary species is consistent with such a response.

Atmospheric VPD and *E* follow a diurnal pattern, being lowest at sunrise and increasing to a maximum at around 15:00 (Hirasawa and Hsiao 1999). In this paper, the two minimum values of VPD matched the report by Hirasawa and Hsiao, but they differed in terms of their maximum values (which appeared at 13:00, Fig. 2*D*). In addition, *E* was also different in its time of appearance in the two species. *F. cirrhosa* peaked at 11:00 while *F. delavayi* peaked at 16:00. These results were controlled by differences in vapour diffusivity. Among factors causing resistance to vapour diffusivity, g_s was the greatest contributing factor. After 10:00, the VPD of *F. cirrhosa* increased sharply while *E* dropped rapidly, which means that g_s of *F. cirrhosa* was environmentally impressible. Stomata closure was more marked in *F. cirrhosa* than in *F. delavayi*. g_s of *F. delavayi* was 4 times higher at 8:00 than at 14:00, and C_i (Fig. 2*E*) was 1.5 times higher. g_s of *F. cirrhosa* was 3.2 times higher at

8:00 than at 14:00, and C_i was 1.8 times higher. Partial stomata closure occurred in both species. WUE may be improved by partial stomata closure such that C_i is just sufficient for saturation of P_N while the rate of water loss is significantly lowered (Kang *et al.* 1998). The decrease in P_N induced by stomata closure was more evident than the decrease observed in C_i, suggesting that non-stomata limitation of photosynthesis also occurs (Nunes *et al.* 2007). Stomata closure is a drought avoidance response that allows for leaf water content maintenance (Ludlow 1980). However, the consequent decrease of C_i (Fig. 2*E*) limits photosynthesis (Cornic 2000, Flexas and Medrano 2002) and results in the afternoon depression of photosynthesis.

F. cirrhosa had low LWC (74.56 %, unpublished data) but had a high WUE compared to *F. delavayi* (Fig. 2*F*). This may be associated with higher productivity under drought (Munns 2005, Chaves *et al.* 2004). WUE is associated with many underlying and interrelated traits and is affected by environmental factors (Kozi 2006). Contributions of these factors to WUE are accumulative and complex (Wang *et al.* 2003b). Apparent quantum yield is an important parameter necessary to reveal photochemical efficiency. In this experiment, both species showed a high AQY at sunrise and at sunset and a low AQY in the afternoon (Fig. 2*G*). This indicates that both species encountered photo-inhibition and partial stomata closure under irradiance.

From diurnal changes of different parameters, we can see that the two fritillary species have well-adapted eco-physiological abilities. *F. cirrhosa* had advantages in production and *F. delavayi* was better adapted to strong irradiance and drought.

Light response curves: *F. cirrhosa* had a higher P_N than *F. delavayi*, but a larger standard error of mean among individual plants (Fig. 3). The P_{max} of *F. cirrhosa* was 30 % higher than *F. delavayi* ($p<0.05$). Results for AQY showed a comparable difference between the two species (27.3 %, $p<0.01$), which was beneficial for photosynthetic yield (Table 1). LSP in *F. delavayi* was by 147 μmol m^{-2} s^{-1} higher than in *F. cirrhosa*. LCP in *F. delavayi* was by 8.47 μmol m^{-2} s^{-1} lower than in *F. cirrhosa*. This made *F. delavayi* photosynthesize in a wider range of irradiance condition. There were no differences in *E* and R_D.

Correlations between P_N and environmental factors: A correlation between P_N and environmental factors in the leaves of *F. cirrhosa* showed that relative humidity ($p<0.01$), T_{soil} ($p<0.05$) and air pressure ($p<0.01$) had significant effects on P_N, of which only T_{soil} was negatively and significantly related with photosynthetic yield (Table 2). Path analyses showed that relative humidity was the only parameter with the same outcome as the results of the correlation. Air temperature and PPFD were the two factors most strongly related to P_N, and both direct path coefficients were higher than their respective correlation coefficients. When an indirect path coefficient analysis was carried out, very low direct path coefficients were found for CO_2 concentration, relative humidity, T_{soil}, soil humidity and air pressure, while the correlation coefficient of T_{air} was the highest. However, the effect of relative humidity of P_N resulted mainly from T_{air}. From the results of two analyses, we found variance between them. T_{air} and PPFD had insignificant effects on P_N in correlation analysis while showed a reverse result in path analysis. The main reason was that original data of the two factors showed an insignificant change from 9:00 to 17:00. So, simple correlation coefficients did not reflect the important relationship between P_N and the two factors. Although air pressure and T_{soil} had an important influence on P_N, they might be only exhibited by pure data and the real contributing factor was T_{air}. Therefore, we deduced an elementary outcome: T_{air}, PPFD and relative humidity were the three primary environmental factors that affected the gas exchange of *F. cirrhosa* under field conditions.

A correlation between P_N and environmental factors in the leaves of *F. delavayi* showed little difference from *F. cirrhosa*, although there were four environmental factors related significantly to P_N (Table 3). The sequence from high to low was relative humidity ($p<0.01$), T_{soil} ($p<0.05$), CO_2 concentration ($p<0.05$) and air pressure ($p<0.05$), of which relative humidity, T_{soil} and air pressure were the influencing factors shared by *F. cirrhosa* and *F. delavayi*. These results indicate that these three factors might be the growth limiting factors we wanted to find. Path analysis showed that T_{air}, PPFD, T_{soil} and air pressure were the four primary determined factors. PPFD and air pressure contributed to P_N directly, while the other two factors affected P_N indirectly. We found that T_{soil} and T_{air} were closely linked with air pressure. In both analyses, T_{soil} showed significant effect.

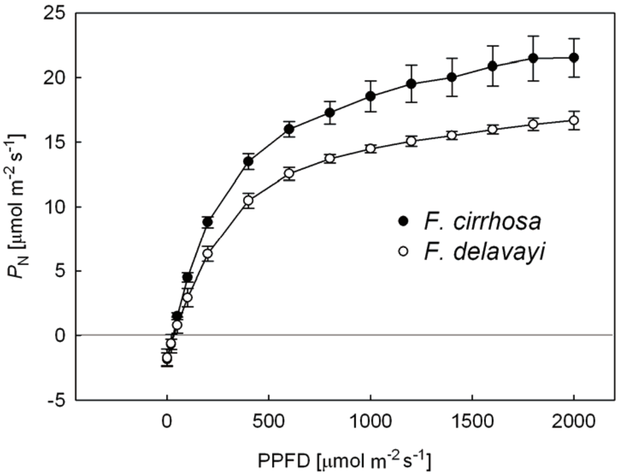

Fig. 3. The photosynthesis (P_N)-irradiance (PPFD) response curves in leaves of *F. cirrhosa* and *F. delavayi*. Each value represents the means ± SE (n=6).

Table 1. Differences in photosynthetic parameters between *F. cirrhosa* and *F. delavayi.* Maximum photosynthetic rate (P_{max}), transpiration rate (E), dark respiration (R_D), apparent quantum yield (AQY), light saturated point (LSP), light compensated point (LCP). Each value represents the mean ± SD (n=6).

Parameters	*F. delavayi*	*F. cirrhosa*	Significance
P_{max} [μmol m^{-2}s^{-1}]	16.13±0.53	20.98±3.33	0.012
E [μmol m^{-2}s^{-1}]	3.15±0.67	4.11±1.06	0.32
R_D [μmol m^{-2}s^{-1}]	1.72±0.66	1.82±0.97	0.84
AQY [mmol mol^{-1}]	0.044±0.003	0.056±0.005	0.003
LSP [μmol m^{-2}s^{-1}]	1728±164	1581±330	0.07
LCP [μmol m^{-2}s^{-1}]	31.7±15.90	23.23±12.24	0.52
r^2	0.987±0.003	0.997±0.001	0.004

Table 2. Correlation and path analyses between photosynthetic rate and environmental factors in leaves of *F. cirrhosa.* X_1 (air temperature), X_2 (CO_2 concentration), X_3 (relative humidity), X_4 (PPFD), X_5 (soil temperature), X_6 (soil humidity), X_7 (air pressure); * and ** indicate a significant difference at the 0.05 and 0.01 levels, respectively. The values in bold show significant effect.

	Pearson correlation coefficient	Direct path coefficient	Indirect path coefficient						
			$\to X_1$	$\to X_2$	$\to X_3$	$\to X_4$	$\to X_5$	$\to X_6$	$\to X_7$
X_1	−0.520	**−1.771**		0.455	−0.595	0.903	0.302	−0.031	0.217
X_2	0.400	−0.525	1.537		0.555	−0.645	−0.330	0.022	−0.214
X_3	0.721**	**0.660**	1.597	−0.441		−0.531	−0.341	0.019	−0.242
X_4	0.135	**1.300**	−1.230	0.260	−0.269		0.040	−0.033	0.067
X_5	−0.674*	0.411	−1.301	0.421	−0.547	0.125		−0.012	0.229
X_6	0.124	0.053	1.037	−0.222	0.236	−0.823	−0.097		−0.060
X_7	0.751**	−0.253	1.521	−0.445	0.632	−0.345	−0.372	0.013	

Table 3. Correlation and path analysis between photosynthetic rate and environmental factors in leaves of *F. delavayi.* X_1 (air temperature), X_2 (CO_2 concentration), X_3 (relative humidity); X_4 (PPFD), X_5 (soil temperature), X_6 (soil humidity), X_7 (air pressure); * and ** indicate a significant difference at the 0.05 and 0.01 levels, respectively. The values in bold show significant effect.

	Pearson correlation coefficient	Direct path Coefficient	Indirect path coefficient						
			$\to X_1$	$\to X_2$	$\to X_3$	$\to X_4$	$\to X_5$	$\to X_6$	$\to X_7$
X_1	−0.465	**−1.534**		−0.089	−0.523	0.841	−1.297	0.170	1.967
X_2	0.567*	0.101	1.354		0.574	−0.438	1.594	−0.126	−2.490
X_3	0.769**	0.693	1.157	0.083		−0.059	1.797	−0.084	−2.818
X_4	0.143	**1.266**	−1.018	−0.035	−0.032		0.084	0.172	−0.294
X_5	−0.638*	**−2.013**	−0.988	−0.080	−0.619	−0.053		0.061	3.053
X_6	0.152	−0.288	0.907	0.044	0.202	−0.758	0.424		−0.380
X_7	0.562*	**−3.140**	0.960	0.080	0.622	0.118	1.958	−0.035	

Table 4. The differences in chlorophyll fluorescence parameters between *F. cirrhosa* and *F. delavayi*. Each value represents the mean ± SD (n=10). *Data measured before dawn and **in the afternoon (13:00~15:00)

	$F_v/F_m{}^*$	$F_v/F_m{}^{**}$	F_v'/F_m'	Φ_{PSII}	q_P	NPQ	ETR
F .cirrhosa	0.83±0.03	0.77±0.03	0.41±0.06	0.22±0.04	0.41±0.13	1.98±0.23	123±29
F. delavayi	0.82±0.02	0.79±0.01	0.43±0.01	0.23±0.01	0.50±0.03	2.41±0.14	134±9.0

Wild *F. delavayi* lives beyond 4000 m where T_{soil} was always lower than 13 °C. In the measurement site, the maximum T_{soil} reached 23.8 °C and changed greatly from 6:00 to 18:00. That's why it showed a significant effect negatively. As such, PPFD, air pressure, T_{soil} and T_{air} (negative correlation) were elementarily regarded as growth limiting factors for *F. delavayi*.

Air pressure had a significant impact directly or indirectly on photosynthesis in this experiment, which looked impossible at constant altitude. However, repeated experiments have shown that air pressure differs with time of day, which might be due to extreme climate changes in the altiplano. There are few indications in the literature that environmental factors during growing periods can, in fact, have a marked influence on P_N by air pressure (Shen 1990). In addition, we don't deny that the relationship between air pressure and photosynthetic rate was only coincident in pure data. This needs to be further studied in future studies.

According to these results, we may regulate the growth of *F. cirrhosa* and *F. delavayi* manually. We can conclude from our data that environmental factors react with each other. Controlling one or two factors may reduce the damage caused by other stresses. Wild *F. cirrhosa* grows under shady conditions. We copied the surrounding irradiance of wild *F. cirrhosa* (50 % of full light) and irrigated it with a relative soil humidity of 40–60 %. T_{air} and T_{soil} decreased by 3 °C, and the maximum P_N increased by 31.1 %. F_v/F_m, F_v'/F_m' , q_p , ETR, and Φ_{PSII} were all up-regulated (Li and Chen 2008).

Chl fluorescence measurements were performed on dark-adapted attached leaves of *F. cirrhosa* and *F. delavayi* growing in a field in Kangding County. The relative differences between the species are presented in Table 4. There were no significant differences in F_v/F_m measured before dawn, and both values were greater than 0.80. This indicates that the two species were either not stressed or were mostly unaffected by stress (Björkman *et al.* 1987). The values of F_v/F_m measured in the afternoon (13:00~15:00) showed diurnal transient decreases (7.2 % and 3.7 %, respectively) in the two species, which is a general feature of photosynthesis in natural environment (Veres *et al.* 2002). However, their values were still higher. We concluded that environmental factors were not the primary cause for their extinction. In the strong irradiance, the relative parameters (F_v'/F_m', ETR, Φ_{PSII}) of *F. delavayi* were all higher than *F. cirrhosa*, which proved that *F. delavayi* was more tolerant to high T_{air} and irradiance stress. A high q_P was beneficial to increase electron transport and quantum yield, which was confirmed by the higher F_v'/F_m' and ETR of *F. delavayi* relative to *F. cirrhosa*. NPQ represented the energy dissipated as heat (Veres *et al.* 2006), and redundant energy becomes a potential source of damage to the photosynthetic apparatus (Melis 1999). *F. delavayi* had a high NPQ, which could protect photosynthetic reaction centres from damage induced by irradiation. Thus, compared with *F. cirrhosa*, *F. delavayi* was better adapted for thermal dissipation under the same irradiance.

In this experiment, the data showed that T_{air} was negatively correlated with P_N, which seemed unreasonable in an area of high elevation. This was because the highest temperature at the research station reached 35 ºC, beyond the optimal temperature (12–26 ºC) of the species' natural habitats. In their original habitats, the environment was shady and humid for *F. cirrhosa* and cool for *F. delavayi* beyond 4200 m. When the two species were introduced at the research station, no shading treatments were performed and watering was infrequent, which lead to a high T_{air}.

Conclusion

Our study revealed for the first time that *F. cirrhosa* and *F. delavayi* could be efficiently acclimated under field conditions. A small reduction in F_v/F_m was observed in high irradiance. Although the two species showed afternoon depression of photosynthesis, values of P_N in both species were positive when PPFD reached beyond 2100 μmol m^{-2} s^{-1} and under high temperature stress. We concluded that the two species could grow well in research station and environmental factors might not be the main cause of the wild extinction of the two species. *F. cirrhosa* was favoured in photosynthetic yield but not under irradiance and drought stresses compared with *F. delavayi*. *F. delavayi* was more adapted to a wide range of irradiances and temperatures. We can choose the suitable species to cultivate according to our aims and conditions of growth.

Correlation between the photosynthetic rate and environmental factors in the leaves of *F. cirrhosa* revealed that the three factors of primary importance were air pressure > relative humidity > T_{soil}. The factors in *F. delavayi* were relative humidity > T_{soil} > CO_2 concentration > air pressure. Path analysis showed that T_{air} (negative correlation), PPFD and relative humidity were the three primary limiting factors influencing the gas exchange of *F. cirrhosa*, where relative humidity varied with T_{air}. Limiting factors for *F. delavayi* were PPFD, air pressure (negative correlation), T_{soil} (negative correlation) and T_{air} (negative correlation).

From these results, relative humidity, PPFD and air temperature should be carefully considered in cultivation.

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