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Characteristics of sun- and shade-adapted populations of an endangered plant *Primulina tabacum* Hance

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Abstract

Primulina tabacum Hance is an endangered perennial herb distributed in calcium-rich and nitrogen-limited soil of the karst limestone areas in southern China. The morphological, ultrastructural, and physiological traits were determined for *P. tabacum* populations growing in three different environment conditions: twilight zone of a cave (site TZ, extremely low light intensity), at a cave entrance (site EZ, low light intensity), and in an open area (site OA, high light intensity). At site OA, *P. tabacum* plants were exposed to high light (635 μmol m^{-2} s^{-1} of mean daily photosynthetically active radiation) with drought stress, and expressed traits to minimize light capture and water loss. Compared to plants at sites EZ and TZ, those at site OA had thicker leaves with higher densities of stomata and pubescence, higher palisade/spongy ratio, higher light-saturated rate of net photosynthetic rate (P_{max}), higher biomass, higher non-photochemical quenching coefficient (NPQ), and higher light saturation point (LSP) but fewer grana per chloroplast and less thylakoid stacking per granum. In contrast, *P. tabacum* growing at the cave vicinities: EZ (mean daily irradiance 59 μmol m^{-2} s^{-1}) and TZ (mean daily irradiance 11 μmol m^{-2} s^{-1}) showed typical shade-adapted characteristics for optimum light capture. The presence of sun- and shade-adapted characteristics indicates that *P. tabacum* has different strategies to cope with different environments but whether these strategies reflect genetic selection or phenological plasticity is yet to be determined. Such variability in physiological and morphological traits is important for the survival of *P. tabacum* in heterogeneous light conditions.

Additional key words: cave microenvironment; chloroplast ultrastructure; ecophysiological trait; light adaptation; morphological structure; *Primulina tabacum* Hance.

Introduction

The conservation of rare and endangered plants encounters increasing attention. Several previous studies on endangered plants have already focused on their biological characteristics or the causes of endangerment. However, few studies have shed some light on how the ecophysiological and structural characteristics of plants, especially their photosynthetic characteristics and ecophysiological requirements, are affected by habitat (Matos *et al*. 2009, Ren *et al*. 2010b).

P. tabacum Hance (Gesneriaceae) is a critically

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Abbreviations: AP – available phosphorous; C_a – air CO_2 concentration; C_i – internal CO_2 concentration; Car – carotenoid; CCP – CO_2 compensation point; Chl – chlorophyll; CSP – CO_2 saturation point; E – transpiration rate; EZ – entrance zone; F_0 – minimal fluorescence of dark-adapted state; F_m – maximal fluorescence of dark-adapted state; F_0' – minimal fluorescence of light-adapted state; F_m' – maximum fluorescence of light-adapted state; F_s – steady-state fluorescence yield; g_s – stomatal conductance; I – the intensity of photosynthetically active radiation; LAR – leaf area ratio; LCP – light compensation point; LMA – leaf mass per unit area; LMF – leaf mass fraction; L_s – stomatal limitation; LSP – light saturation point; NPQ – non-photochemical quenching; OA – open area; PAR – photosynthetically active radiation; P_{max} – light- saturated net photosynthetic rate; P_N – net photosynthetic rate; q_P – photochemical quenching; $rETR_{max}$ – relative maximum electron transport rate; RH – air relative humidity; R_d – non-photo-respiratory mitochondrial CO_2 release; R_D – dark respiration; SOM – soil organic matter; SWC – soil water content; T_a – air temperature; TN – total nitrogen content; TZ – twilight zone; V_{cmax} – maximal carboxylation rate of Rubisco; VPD – vapour pressure deficit; WUE – water-use efficiency; Φ – apparent quantum efficiency; Φ_{PSII} – effective quantum yield of PSII photochemistry.

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endangered perennial herb endemic to the semitropical karst areas of China. The population size of *P. tabacum* has drastically decreased during the last three decades mainly because of increasing anthropogenic disturbances such as increased tourism exploration and excavation of lime stone (He and Li 2005, Ren *et al*. 2010a,b). The species get already listed among the 'first class protected key wild plants of China' (Peng and Chen 2002) and presently, it is found only at eight sites in the region between Guangdong and Hunan Provinces (Ren *et al*. 2010b).

Although many researches have been focused on the adaptation of fauna, fungi, and bacteria to cave ecosystems (Culver *et al*. 2000, Northup and Lavoie 2001, Cuezva *et al*. 2009, Biswas 2010), little is known about the adaptation of higher plants to the vicinity of caves, especially in the ecotone of the caves. The perpetual darkness, high humidity, low air flow, and higher CO_2 concentrations altogether make the cave ecotone a unique niche (Krajick 2001, Wynne and Pleytez 2005, Biswas 2009). Survival in such habitats undoubtedly requires many physiological and structural adjustments.

A single cave may offer different physical environment and the changes in vegetation in different zones of the cave vicinity are mainly influenced by photosynthetically active radiation (PAR), air temperature (T_a), air CO_2 concentration (C_a), and moisture (Allan and Zhang 2001, Ren *et al*. 2010b). In the studied cave vicinity, the distribution of *P. tabacum* was seen to flourish successfully in the ecotone (entrance to twilight zones). A previous study by Ren *et al*. (2003) found that *P. tabacum* grew slowly, with a maximum growth rate of <30 g yr^{-1}(fresh mass). In the cave vicinity this species was largely restricted to the cave ecotone and the population density was largest at entrance zone, whereas the individuals in the deep cave were pale and spindly because of the lack of light.

Although *P. tabacum* is skiophilous species, it seems to grow safely in the ecotone of the limestone cave (Ren *et al.* 2010b), however in the field ecological studies, we found that some populations occur in limestone open sites in Xiaobeijiang and Xiaguancun. In these habitats, the soil is shallow and rocky, leading to quick drainage and low water-holding capacity. Thus except during the rainy season, the *P. tabacum* populations in these open sites clearly experience greater sunlight and drought stress than those grown in the cave vicinity as a result of higher irradiance, temperature and depleted soil water buffering.

Understanding the variabilities in morphology, structure, and physiology are important to know how a plant species is able to grow in different habitats, which ultimately will also guide the selection of suitable habitats for the possible reintroduction of the species. Till date, most of the studies on *P. tabacum*, however, have focused on taxonomy (Flora of China Editorial Committee 1990), ecological and biological characteristics (Ren *et al.* 2003), pollen morphology (Cao *et al.* 2007), and genetic diversity (Ni *et al.* 2006, Wang *et al.* 2009). The ecophysiological or morphological characteristics of this species have remained almost untouched. To enhance our understanding of the ecological adaptations or plasticity of *P. tabacum*, we have compared differences among populations growing in three karst habitats with different light intensities. The parameters measured were photosynthetic rates, photosynthetic pigment contents, chlorophyll (Chl) fluorescence, leaf and stem morphology, and chloroplast ultrastructure. The present study was aimed to answer: (*1*) Do the structural or physiological characteristics differ among *P. tabacum* populations adapted in different habitats? (*2*) Do the habitats with different light conditions impart any differences in the structural or physiological characteristics of *P. tabacum* adaptations?

Materials and methods

Physical characteristics of studied cave zones: The studied cave site with a subterranean river is located at Jiuyi Mountain. The mountain belongs to a limestone outcrop covered by an evergreen forest at its top. The floor in the cave is rocky and with scattered thin soil layer on it. The cave consists of a large entrance hall about 30 m wide and 20 m tall, which goes steeply down, and a horizontal gallery which reaches the underground river some 200 m away. The cave can be divided into three groups based on their dependence on light resource (Poulson and White 1969, Howarth 1983): (*1*) the entrance zone, which is characterized by reduced light levels (relative to the area outside of the cave), increased relative humidity, moderate temperature fluctuations, and increased CO_2 concentration; (*2*) the twilight zone in which relative darkness prevails and temperature fluctuates less than at the entrance; and (*3*) the dark zone in which total darkness and constant temperature prevail. As compared with dark zone, the presence of light in entrance and twilight zone allows the growth of photosynthetic organisms that result in an increase of resource availability and richness of species, thus the region between entrance and twilight zone in the cave can be characterized as ecotone that shows gradients of biological and physical modifications, creating a transition zone between aboveground and the underground ecosystems (Prous *et al*. 2004). In this research, we found that the distributions of *P. tabacum* are limited to the ecotone where environmental variables are under significant influence from the external environment.

Study sites and materials: Three study sites were

selected in Ningyuan County in southern Hunan Province. The area receives an annual average total precipitation of 1,600 mm, and the mean annual temperature is 18.4°C. The wettest month is July and the driest one is January. One of the sites was an open area (OA) at Xiaguancun village (N 25°27′062″, E 112°02′416″). The second site was the entrance zone (EZ) of Zixiayan cave, at an altitude of 300 m on Jiuyi Mountain (N 25°20′941″, E 111°58′498″). The third site was in the twilight zone (TZ) about 20–30 m distant from the entrance of the Zixiayan cave. The *P. tabacum* plants at the EZ and TZ sites belong to the same population. Because the OA site is 32 km away from the Zixiayan cave, the *P. tabacum* at the OA site is considered an independent population.

Soil physical and chemical characteristics of the three sites: Soil samples were collected in July 2008 (wet season) and January 2009 (dry season). Because the soil layer around the study sites were very thin, soil samples were collected with a 5-cm diameter soil cores to a depth of 0.5–2.0 cm after the litter and humus layer were removed. Four soil cores were taken randomly from each quadrat (three quadrats per site). Cores from each quadrat were mixed, air-dried and sieved in the laboratory for analysis the physical and chemical characteristics. Soil chemical properties (including pH, soil organic content, total nitrogen content, available phosphorus, and soil exchangeable Na, K, Ca, and Mg) were analyzed by standard methods (Liu 1996, Duan *et al*. 2008). Soil water content (SWC) of each sample was determined gravimetrically by weighing before and after the samples were dried in an oven at 105°C for 12 h.

Microclimate and leaf gas-exchange measurements: The quantity of solar irradiance received by plants differed among the three sites. Environmental factors (*i.e.*, PAR, T_a, RH, and C_a) were measured by sensors on a portable photosynthesis system (*LI-6400*, *Li-Cor*, Lincoln, NE, USA) for 3 days at each site in July 2008 (wet season) and January 2009 (dry season).

Net photosynthetic rate (P_N), stomatal conductance (g_s), intercellular CO_2 concentration (C_i), and transpiration rate (E) were measured in attached leaves on sunny days (three sunny days in July 2008 and three sunny days in January 2009) from 10:00 to 16:00 at 2-h intervals with *LI-6400* system. Water-use efficiency (WUE) was calculated as P_N/E (Dewar 1997). Stomata limitation (L_s) was calculated as $1 - C_i/C_a$ (Berry and Downton 1982). Ten plants at each site were randomly selected, and to reduce variation between samples, only mature and healthy leaves were measured and the same leaves were resampled throughout the day. To compare the P_{max}, light compensation point (LCP), and LSP between different sites in consistent condition, the CO_2 concentration (380 μmol mol^{-1}) and temperature (25°C) were maintained uniformly in *Li-6400* leaf champ, P_N–PAR response curve was determined at PAR values ranging from 0 to 2,000 μmol m^{-2} s^{-1}. The net photosynthesis–CO_2 response curve was measured at CO_2 concentrations of 50–2,000 μmol mol^{-1} at 25°C with a PAR value slightly over LSP. The PAR and CO_2 curves were composites of five individual measurements at each site.

The dependence of P_N on PAR of leaves was fitted by the Walker (1989) model of leaf photosynthesis:

$$P_N = \frac{\Phi I + P_{max} - \sqrt{(\Phi I + P_{max})^2 - 4\theta\Phi I P_{max}}}{2\theta} - R_D \qquad (1)$$

where I is the intensity of PAR, P_{max} is the light-saturated P_N, Φ is the apparent quantum efficiency, θ is the curvature factor of the nonrectangular hyperbola, and R_D is the dark respiration. LCP was calculated when the photosynthetic rate approached zero, LSP was calculated as the lowest value of PAR for which photosynthesis reached 90% of P_{max}. Parameters of the model were calculated by the nonlinear estimation module of *SPSS 13.0 for Windows* (*SPSS*, Chicago, IL, USA).

The dependence of P_N on C_i was fitted using the model described by Farquhar *et al*. (1980) and Farquhar and von Caemmerer (1982). At low C_i and high irradiance, the maximal carboxylation rate of Rubisco (V_{cmax}) was calculated (ignoring the CO_2 diffusion limitation within the leaf) as:

$$V_{cmax} = (A + R_d)\frac{C_i + K_c(1 + O/K_o)}{C_i - \Gamma} \qquad (2)$$

where A is the rate of CO_2 assimilation; K_c and K_o are Michaelis-Menten constants for CO_2 and O_2, respectively; C_i is the intercellular CO_2 concentration; Γ is the CO_2 compensation concentration in the absence of non-photorespiratory mitochondrial CO_2 release (R_d); O is the oxygen partial pressure; and R_d was determined from the P_N-C_i curve near the compensation concentration with the rate at C_i = Γ. The values derived for *Nicotiana tabacum* by von Caemmerer *et al*. (1994) are K_c = 40.4 Pa, K_o = 24.8 kPa, Γ = 3.69 Pa, and O = 20.5 kPa. The following parameters were obtained by fitting the equation and the measured P_N-C_i curve: V_{cmax}, CO_2 saturation point (CSP), and CO_2 compensation point (CCP).

Chl fluorescence of photosystem II (PSII) was measured with a portable, pulse-amplitude, modulated fluorometer (*PAM-2100, Walz*, Effeltrich, Germany). Before measurement, the leaves were kept in leaf clamps for 30 min of dark adaptation. Minimal fluorescence of dark-adapted leaf (F_0) was measured under a weak modulated radiation (0.5 μmol m^{-2} s^{-1}). The maximal fluorescence of dark-adapted leaf (F_m) was induced by 0.8-s pulse of saturating light (2,700 μmol m^{-2} s^{-1}). For measurement of fluorescence quenching components, the sample was continuously irradiated with an actinic light of 185 μmol m^{-2} s^{-1}. The steady-state fluorescence (F_s) was then recorded within 5 min after a second saturating pulse was

imposed to determine the maximum fluorescence of light-adapted state (F_m'). At the end of measurement, a weak 5-s far-red light was used to determine the minimal fluorescence of light-adapted state (F_0'). According to Souza *et al*. (2004) and Han *et al*. (2005), the maximum quantum yield of PSII photochemistry $F_v/F_m = (F_m - F_0)/F_m$, the photochemical quenching coefficient $q_P = (F_m' - F_s)/(F_m' - F_0')$, non-photochemical quenching coefficient $NPQ = (F_m - F_m')/F_m'$, and effective quantum yield of PSII photochemistry $\Phi_{PSII} = 1 - F_s/F_m'$.

PAM-2100 was also used to determine the relative maximum electron transport rate ($rETR_{max}$) derived from the rapid light curve (RLC). To generate RLC, leaves were irradiated with a series of actinic light intensities (91, 94, 160, 240, 346, 522, 707; 1,161; 1,781 μmol m^{-2} s^{-1}) for 10 s, always finishing with a saturating pulse after each level of illumination. The relative electron transport rate $rETR = 0.84 \times 0.5 \times \Phi_{PSII} \times I$, where I is the intensity of photosynthetically active radiation and the $rETR_{max}$ are maximum electron transport rate values calculated at saturating actinic irradiances.

Anatomical and morphological measurements: Total plant biomass and leaf samples were oven-dried (70°C, 48 h) to constant mass and weighed. Leaf area was determined using a *LI-3000* leaf area meter (*LI-COR*, *Lincoln*, Nebraska, USA). Leaf mass per unit area (LMA) was calculated from leaf dry mass and leaf area. Leaf area ratio (LAR) was calculated as the ratio of leaf area to total plant biomass. The leaf mass fraction (LMF) was calculated as the ratio of leaf mass to total aboveground plant mass.

For anatomical measurements, plant material was fixed in a solution of 70% ethanol: 40% formaldehyde: glacial acetic acid (90:5:5, v/v/v), dehydrated, and embedded in paraffin wax (Ruzin 1999). Leaf and stem tissues were sectioned (3–5 μm thick) using a sliding microtome, and sections were stained with both safranin and hematoxylin. All sections were observed and photographed with microscope (*AX70, Olympus*, Tokyo, Japan).

Samples for scanning electron microscope (SEM) observation of leaf stomata and pubescence were fixed in 4% glutaraldehyde solution, vacuum-infiltrated for 2 h, stored in a refrigerator, rinsed with 0.1 mol L^{-1} phosphate saline buffer (PSB), and dehydrated through an alcohol series. Isoamyl acetate was used to replace the alcohol before the samples were freeze-dried in a freeze-drying device (*JFD-310, JEOL*, Japan). After being coated with a gold-palladium mixture in a sputter coater (*JFC-1600, JEOL*, Japan), the prepared samples were examined with the SEM (*JSM-6360LV, JEOL*, Japan), and the images were digitally recorded.

Chloroplast ultrastructure: The central part of the leaf disc was cut into pieces (5 mm × 1 mm) , fixed with 4% glutaraldehyde, and rinsed with 0.1 M sodium dimethyl-arsenate buffer. Afterward, samples were fixed with 1% OsO_4 in the same buffer, dehydrated in a gradient of ethanol solution, and embedded in EP 812 resin. Ultrathin sections were prepared with an ultramicrotome (*Reichert Ultracut S*, Germany) and stained with 2% aqueous uranyl acetate followed by 6% lead citrate. Electron micrographs were obtained with a transmission electron microscope (*JEM-1010*, *JEOL*, Japan).

Pigment content: Chl and carotenoid (Car) contents were measured on the same leaves that were tested for photosynthesis and Chl fluorescence. Leaf disks (6 mm in diameter) were extracted with 80% acetone in the dark for 5 days. Absorption of the extracted solutions was measured with a *UV-Vis* spectrophotometer (*Unico, UV-3802*, China). Total Chl, Chl *a*, Chl *b*, and Car were calculated on a leaf-area basis according to Lin *et al*. (1984).

Macronutrient analyses: Harvested plants were separated into leaves, stems, and roots and dried for at least 72 h at 65°C until constant mass was reached. N content was determined by the micro-Kjeldahl method. After wet digestion with nitric and perchloric acid, the concentrations of P were determined through molybdenum-antimony colorimetry, and the concentrations of Mg, Ca, K, and Na were determined by atomic absorption spectrometry (*AAS, GBC932AA*, Australia).

Statistical analysis: The effects of season and site on the environmental factors (PAR, RH, T_a, C_a, and SWC) and physical traits of *P. tabacum* were assessed using two-way *ANOVA*. The effect of site on structural traits of *P. tabacum* and soil physical and chemical traits were assessed by one-way *ANOVA*. Mean values of the macronutrient content of *P. tabacum* in the aboveground and belowground were subjected to a Student's *t*-test. Variables were $\log_{10}$ or arcsine square-root transformed when they did not satisfy normality assumptions. Multiple comparison analyses (LSD) were used when *ANOVA*s were significant at $\alpha = 0.05$. All statistical tests were performed using *SPSS 13.0 for Windows* (*SPSS*, Chicago, IL, USA).

Results

Environmental traits and macronutrient content of *P. tabacum*: The diurnal mean value of PAR, RH, T_a, and C_a (10:00–16:00) differed ($p<0.05$) among the three sites (Table 1). At the OA site, which was characterized by high solar irradiance, PAR during the experimental day was about 10 times greater than that at site EZ and about 50 times greater than that at site TZ. Based on data in Table 1, PAR was the major difference among three sites, and PAR probably explained the differences in the other environmental factors. For example, T_a was higher and

Table 1. Habitat characteristics of the three sites (OA, EZ, and TZ) in the dry and wet seasons. Values for intensity of PAR (I), air CO_2 concentration (C_a), air temperature (T_a), air relative humidity (RH), and soil water content (SWC) are the means (± SD, n = 40) of data collected from 10:00 to 16:00. EZ – entrance zone; TZ – twilight zone; OA – open area. Within each row, different *capital letters* within the same season indicate significant differences between sites, while different *lowercase letters* within the same site indicate significant differences for seasons at $p<0.05$.

Parameter	OA		EZ		TZ	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
I [μmol m^{-2} s^{-1}]	635.4 ± 146.4^{Aa}	476.1 ± 56.0^{Aa}	59.2 ± 25. 7^{Ba}	84.5 ± 44.1^{Ba}	11.8 ± 9.1^{Ca}	9.4 ± 3.1^{Ca}
C_a [μmol mol^{-1}]	404.7 ± 5.9^{Aa}	361.2 ± 1.9^{Bb}	405.0 ± 1.7^{Aa}	599.6 ± 31.3^{Ab}	410.2 ± 4.9^{Aa}	669.7 ± 53.5^{Ab}
T_a [°C]	24.5 ± 3.6^{Ab}	31.2 ± 1.8^{Ab}	10.6 ± 0.75^{Ba}	27.3 ± 1.2^{Bb}	10.5 ± 1.2^{Ba}	25.6 ± 1.1^{Bb}
RH [%]	22.1 ± 4.9^{Bb}	60.1 ± 10.2^{Aa}	35.9 ± 4.4^{Ab}	66.7 ± 1.3^{Aa}	40.7 ± 5.1^{Ab}	62.5 ± 2.4^{Aa}
SWC [%]	7.4 ± 0.022^{Cb}	15.3 ± 0.031^{Ca}	22.5 ± 0.005^{Ab}	25.9 ± 0.023^{Ba}	18.8 ± 0.020^{Bb}	27.4 ± 0.047^{Aa}

Table 2. Soil physical and chemical characteristics at three sites. TN – total nitrogen content; SOM – soil organic matter; AP – available phosphorus; K – exchangeable potassium; Ca – exchangeable calcium; Na – exchangeable sodium; Mg – exchangeable magnesium. Values are means (± SD, n = 4). Within each column, means with the same letter are not significantly different at $p<0.05$.

Site	pH	TN [g kg^{-1}]	SOM [g kg^{-1}]	AP [g kg^{-1}]	K [g kg^{-1}]	Ca [g kg^{-1}]	Na [g kg^{-1}]	Mg [g kg^{-1}]
EZ	6.69 ± 0.22^{a}	0.599 ± 0.15^{a}	37.13 ± 11.4^{a}	0.039 ± 0.011^{a}	0.225 ± 0.05^{a}	47.08 ± 2.47^{a}	0.199 ± 0.11^{a}	0.153 ± 0.06^{a}
TZ	7.18 ± 0.15^{b}	0.556 ± 0.10^{a}	38.97 ± 4.10^{a}	0.067 ± 0.036^{a}	0.236 ± 0.04^{a}	47.02 ± 1.71^{a}	0.191 ± 0.09^{a}	0.407 ± 0.09^{b}
OA	7.41 ± 0.03^{b}	0.473 ± 0.05^{a}	24.81 ± 2.63^{a}	0.089 ± 0.037^{a}	0.244 ± 0.07^{a}	47.41 ± 0.56^{a}	0.123 ± 0.02^{a}	1.208 ± 0.51^{c}

Table 3. Macronutrient content of *P. tabacum* averaged across three sites. Note: Nutrient content did not differ among sites, and values in this table are means (± SD, n = 18) of the three sites. AG , BG, and WP indicate aboveground plant parts (including leaf blade, leaf stalk, flower, and flower stalk), belowground plant parts (including subterranean stem and adventitious roots), and whole plant, respectively.

Plant part	N [g kg^{-1}]	P [g kg^{-1}]	N/P	K [g kg^{-1}]	Ca [g kg^{-1}]	Mg [g kg^{-1})	Na [g kg^{-1}]
AG	16. 3 ± 4.03^{a}	2.05 ± 0.72^{a}	7.64 ± 2.42^{a}	5.69 ± 3.59^{a}	59.0 ± 15.17^{a}	2.96 ± 1.54^{a}	0.490 ± 0.35^{a}
BG	13.3 ± 1.90^{b}	1.60 ± 0.43^{b}	8.06 ± 1.38^{a}	4.69 ± 2.00^{a}	60.45 ± 27.56^{a}	2.49 ± 1.68^{b}	0.413 ± 0.186^{a}
WP	14.7 ± 3.54	1.82 ± 0.62	7.85 ± 1.91	5.19 ± 2.87	63.23 ± 22.02	2.73 ± 1.58	0.458 ± 0.279

RH was lower at the OA site probably because that site experienced high irradiance. In addition, because of the lower water retention in the soil at the OA site, SWC was much lower at the OA site than at the cave vicinities (EZ and TZ). The cave vicinities had lower T_a values but higher moisture levels relative to the OA site. During the wet season, CO_2 originates from the soil and enters the cave by degassing from vadose water. Thus, the CO_2 concentrations at the cave sites during the wet season were about two times greater than those in the surface atmosphere. Soil pH at the three sites ranged from 6.7 to 7.4 (Table. 2). Except for pH and exchangeable Mg content, soil chemical characteristics did not differ ($p>0.05$) among the three sites (Table 2). Soil pH was lowest at the EZ site, while Mg content was highest at the OA site. The soil layer at the study sites was very thin, and the contents of soil organic matter (SOM), P, and especially N were lower in the soils at the three sites than in some other regional soils (Lu 1989). The nutrient content of *P. tabacum* did not differ among the sites ($p> 0.05$), and mean values are shown in Table 3. The Ca content (whole plant level) was extremely high (63.2 g kg^{-1}) while the N (14.74 g kg^{-1}), K (5.19 g kg^{-1}), and Na (0.45 g kg^{-1}) contents were low in *P. tabacum*. The average N/P ratio in the above-ground biomass of *P. tabacum* from the three site*s* was 7.6.

Photosynthesis: Parameters associated with photosynthesis of *P. tabacum* differed significantly ($p<0.05$) among the three sites in both seasons (Fig. 1). The low P_N values indicated the low light availability in shade leaves. During the wet season, mean daily P_N values were highest at the OA site, lowest at the TZ site, and intermediate at the EZ site levels (Fig. 1*A*). Similarly P_N, E, and g_s during the wet season were highest at the OA site and lowest at the TZ site (Fig. 1*C*,*E*). In contrast, C_i values during the wet season were about 1.3 times higher at both cave sites than at the OA site (Fig. 1*B*). C_i is usually affected by air CO_2 concentration, stomatal conductance, and the efficiency of CO_2 assimilation during photosynthesis, whereas in this study, the increase in C_i of leaves in cave ecotone habitats could possibly have been caused by the high air CO_2 concentration (Table 1).

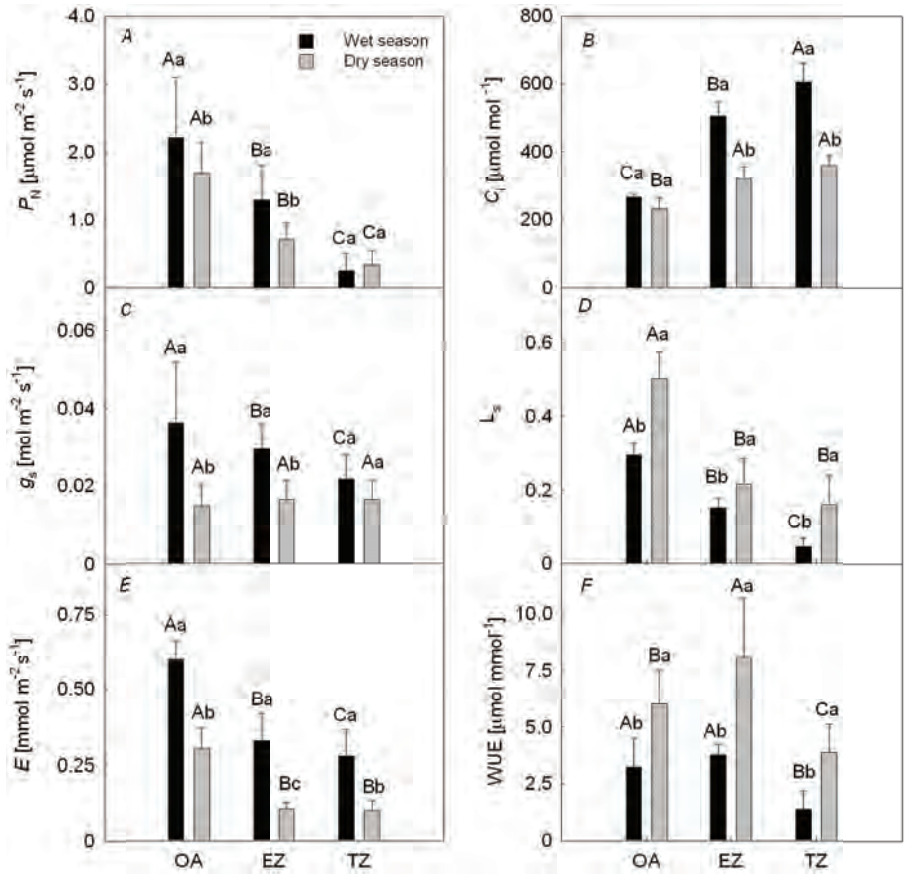

Fig. 1. Net photosynthetic rate (P_N), internal CO_2 concentration (C_i), stomatal conductance (g_s), stomatal limitation (L_s), transpiration rate (E), and water use efficiency (WUE) of *P. tabacum* as affected by site (OA, EZ, and TZ) and season. Note: Values of P_N, C_i, g_s, L_s, and E are the means (± SD, n = 10) of data collected from 8:00 to 16:00 under natural environmental conditions; different capital letters within the same season indicate significant differences between sites, while different lowercase letters within the same site indicate significant differences for seasons at p<0.05.

Table 4. Parameters from light-response curves of *P. tabacum* as affected by site (OA, EZ, and TZ) and season. Note: Values for light compensated point (LCP), light saturated point (LSP), CO_2 compensation point (CCP), CO_2 saturation point (CSP), maximal net photosynthetic rate (P_{max}) and maximal carboxylation rate of Rubisco (V_{cmax}) are means (± SD, n = 5). Within each row, different *capital letters* within the same season indicate significant differences between sites, while different *lowercase letters* within the same site indicate significant differences for seasons at p<0.05.

Parameter	OA		EZ		TZ	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
P_{max} [μmol m^{-2} s^{-1}]	2.22 ± 0.28^{Ab}	4.85 ± 0.59^{Aa}	1.41 ± 0.13^{Bb}	2.51 ± 0.06^{Ba}	0.84 ± 0.06^{Ca}	1.03 ± 0.29^{Ca}
R_D [μmol m^{-2} s^{-1}]	0.65 ± 0.09^{Ab}	0.77 ± 0.02^{Aa}	0.20 ± 0.03^{Bb}	0.49 ± 0.10^{Ba}	0.17 ± 0.08^{Ba}	0.25 ± 0.11^{Ca}
Φ [mol mol^{-1}]	0.049 ± 0.006^{Aa}	0.054 ± 0.005^{Ba}	0.051 ± 0.006^{Ab}	0.072 ± 0.007^{Aa}	0.056 ± 0.003^{Aa}	0.06 ± 0.005^{Ba}
LCP [μmol m^{-2} s^{-1}]	18.7 ± 2.77^{Aa}	13.1 ± 0.91^{Ab}	9.45 ± 2.99^{Ba}	6.14 ± 0.85^{Bb}	6.60 ± 0.13^{Ca}	5.08 ± 1.52^{Ba}
LSP [μmol m^{-2} s^{-1}]	95.54 ± 11.7^{Ab}	138.0 ± 10.24^{Aa}	69.4 ± 8.96^{Ba}	88.8 ± 13.85^{Ba}	40.79 ± 3.78^{Ca}	39.89 ± 12.62^{Ca}
CCP [μmol mol^{-1}]	117.7 ± 13.7^{Aa}	50.5 ± 4.51^{Bb}	89.5 ± 4.48^{Ba}	58.0 ± 5.94^{Bb}	108.0 ± 2.02^{Aa}	78.45 ± 6.98^{Ab}
CSP [μmol mol^{-1}]	676.2 ± 67.2^{Ba}	705.3 ± 80.1^{Ba}	774.4 ± 44.3^{Ab}	845.4 ± 34.3^{Aa}	788.3 ± 33.2^{Ab}	899.0 ± 32.73^{Aa}
V_{cmax} [μmol m^{-2} s^{-1}]	14.5 ± 0.59^{Ab}	18.3 ± 1.19^{Aa}	7.95 ± 0.17^{Bb}	13.3 ± 0.56^{Ba}	5.69 ± 0.46^{Cb}	9.92 ± 1.12^{Ca}

P_N, E, and g_s were lower (p<0.05) in the dry season than in the wet season at the OA and EZ sites. The decreases of P_N were accompanied by increases in L_s (Fig. 1*D*) and decreases in C_i (Fig. 1*B*), indicating that stomata closure could account for the decline in P_N during the dry season. On the other hand, WUE values at all sites significantly increased in the dry season (Fig. 1*F*), apparently because of reduced E (Fig. 1*E*) and P_N (Fig. 1*A*).

Table 4 lists the parameter values calculated from the P_N-PAR response curve and the P_N-CO_2 response curve

Table 5. Photosynthetic pigment content and chlorophyll fluorescence parameters of *P. tabacum* as affected by site (OA, EZ, and TZ) and season. Note: Values are means (± SD, n = 8). Within each row, different capital letters within the same season indicate significant differences between sites, while different lowercase letters within the same site indicate significant differences for seasons at $p<0.05$.

Parameter	OA		EZ		TZ	
	Dry season	Wet season	Dry season	Wet season	Dry season	Wet season
Total Chl [μg cm^{-2}]	19.08 ± 0.86^{Ab}	23.74 ± 2.13^{Ba}	21.97 ± 2.26^{Ab}	27.53 ± 2.48^{Aa}	13.27 ± 0.35^{Ba}	12.73 ± 0.21^{Ca}
Chl *a/b*	2.21 ± 0.14^{Aa}	2.07 ± 0.11^{Aa}	2.26 ± 0.29^{Aa}	1.86 ± 0.08^{ABb}	1.49 ± 0.13^{Ba}	1.65 ± 0.14^{Ba}
Car [μg cm^{-2}]	11.07 ± 1.64^{Aa}	10.94 ± 2.06^{Aa}	9.18 ± 1.43^{Ba}	8.71 ± 2.84^{Ba}	5.37 ± 2.06^{Ca}	3.99 ± 1.12^{Ca}
Car/Chl	0.584 ± 0.14^{Aa}	0.465 ± 0.10^{Ab}	0.426 ± 0.07^{Ba}	0.311 ± 0.03 ^{Bb}	0.351 ± 0.05^{Ca}	0.314 ± 0.09^{Ba}
F_v/F_m	0.769 ± 0.011^{Aa}	0.778 ± 0.006^{Ba}	0.781 ± 0.023^{Aa}	0.793 ± 0.012^{Aa}	0.739 ± 0.013^{Ba}	0.746 ± 0.01^{Ca}
Φ_{PSII}	0.208 ± 0.01^{Ab}	0.560 ± 0.04^{Aa}	0.235 ± 0.04^{Ab}	0.533 ± 0.02^{Aa}	0.136 ± 0.02^{Bb}	0.307 ± 0.04^{Ba}
q_P	0.523 ± 0.02^{Ab}	0.797 ± 0.03^{Aa}	0.565 ± 0.06^{Ab}	0.781 ± 0.19^{Aa}	0.249 ± 0.03^{Bb}	0.302 ± 0.02^{Ba}
$rETR_{max}$ [μmol(e$^-$) m^{-2} s^{-1}]	37.8 ± 1.76^{Ab}	80.8 ± 8.47^{Aa}	20.3 ± 1.75^{Bb}	56.7 ± 12.51^{Ba}	13.3 ± 1.75^{Cb}	24.1 ± 6.21^{Ca}
NPQ	3.26 ± 0.26^{Aa}	2.64 ± 0.55^{Ab}	2.05 ± 0.18^{Ba}	1.43 ± 0.11^{Bb}	2.22 ± 0.19^{Ba}	1.37 ± 0.62^{Bb}

Table 6. Anatomical, ultrastructural, and morphological characteristics of *P. tabacum* collected from three sites (OA, EZ, and TZ). BG/AG – belowground/aboveground biomass ratio; LAR – eaf area ratio; LMA – leaf mass per unit area; LMF – leaf mass fraction. All parameters are normally distributed except the grana thickness and grana per chloroplast, which are log-normally distributed. Values are means ± SD; n = 24 (leaves) for leaf anatomical characteristics, n = 8 (chloroplast) for grana thickness, grana per chloroplast and stacking degree, n = 8 (subterranean stems) for vascular density and stem diameter, n = 8 for total biomass, BG/AG, LMF and LAR, n = 15 for LMA. Within each row, means with the same letter are not significantly different at $p<0.05$.

	Characteristic	OA	EZ	TZ
Anatomical characteristics	Leaf thickness [μm]	272.0 ± 27.3^a	235.8 ± 38.1^b	175.5 ± 30.9^c
	Palisade thickness [μm]	85.02 ± 25.4^a	67.15 ± 15.7^b	42.52 ± 8.9^c
	Sponge thickness [μm]	123.9 ± 24.0^a	118.0 ± 29.3^a	97.4 ± 30.8^b
	Palisade/ spongy ratio [%]	67.79 ± 21.1^a	54.71 ± 9.4^b	46.83 ± 13.9^b
	Upper epidermis thickness [μm]	29.26 ± 5.12^a	30.63 ± 5.45^a	29.11 ± 6.42^a
	Stomatal density [mm^{-2}]	99.83 ± 19.0^a	80.24 ± 22.6^b	37.26 ± 6.1^c
	Pubescence density [mm^{-2}]	74.67 ± 23.6^a	56.85 ± 9.9^b	48.12 ± 18.1^b
	Vascular density of steam [mm^{-2}]	274.8 ± 40.4^a	236.3 ± 26.8^b	230.1 ± 31.0^b
	Stem diameter [mm]	9.64 ± 1.67^a	5.34 ± 1.41^b	4.10 ± 1.23^b
Chloroplast ultrastructure	Grana thickness [nm]	22.50 ± 10.7^c	25.51 ± 12.5^b	31.06 ± 16.8^a
	Grana per chloroplast	29.71 ± 5.70^b	38.56 ± 10.83^a	40.09 ± 6.37^a
	Stacking degree	0.554 ± 0.07^a	0.574b ± 0.06^b	0.582 ± 0.07^b
Morphological characteristics	Total biomass [g]	2.74 ± 0.51^a	2.69 ± 0.52^a	1.24 ± 0.20^b
	BG/AG	0.266 ± 0.047^a	0.164 ± 0.042^b	0.136 ± 0.036^b
	LMF	0.628 ± 0.06^b	0.669 ± 0.04^{ab}	0.687 ± 0.06^a
	LAR	124.5 ± 13.9^c	162.4 ± 8.1^b	248.2 ± 24.7^a
	LMA [g m^{-2}]	48.14 ± 12.5^a	34.72 ± 9.7^b	18.56 ± 8.1^c

(curves not shown) for *P. tabacum* as affected by site and season. Values of P_{max}, V_{cmax}, R_D, LCP, and LSP were higher at the OA site in both dry and wet seasons than at the cave sites. In contrast, CCP and CSP values tended to be lower at the OA site than at the cave ecotone site. Moreover, P_{max} and R_D values were substantially lower at all sites in the dry season than in the wet season: the highest reduction in P_{max} during the dry season occurred at the OA site (reduced by 54.1%) and the lowest one occurred at the TZ site (reduced by 17.9%).

Chl fluorescence and photosynthetic pigment traits: The Chl fluorescence parameters F_v/F_m, Φ_{PSII}, q_P, NPQ, and $rETR_{max}$ were lower at the TZ site than at the other two sites (Table 5), indicating that the adaptation to long-term exposure to extreme shade led to lower activity of PSII photochemistry and electron transport rate. With the increase in light intensity at the EZ and OA sites, NPQ and $rETR_{max}$ increased, and the increase was greater at the OA site, where NPQ was 92% higher than at the TZ site.

Φ_{PSII}, q_P, $rETR_{max}$, and NPQ differed greatly between dry and wet seasons (Table 5). Φ_{PSII}, q_P, and $rETR_{max}$ were greater in the wet season than in the dry one but NPQ was greater in the dry season than in the wet one. The lower PSII activity in the dry season could have resulted from drought stress.

The order of total Chl content per unit leaf area

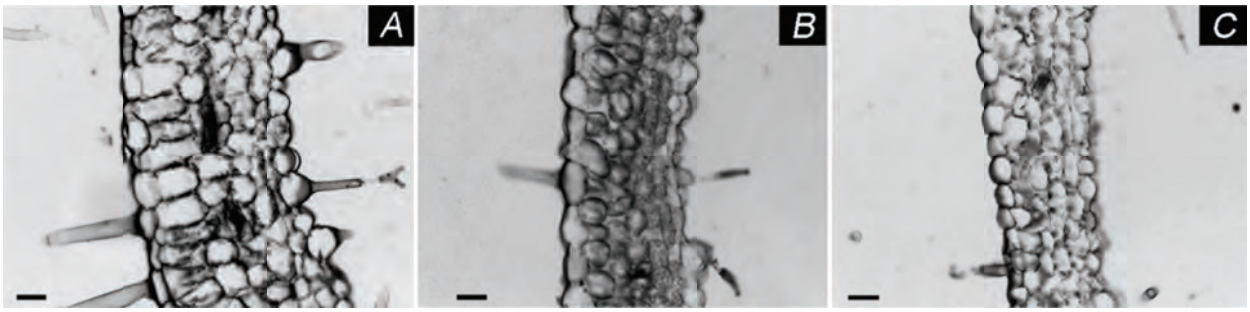

Fig. 2. Transverse sections of *P. tabacum* leaves from the open area (OA) site (*A*), the entrance zone (EZ) site (*B*), and the twilight zone (TZ) site (*C*). *Bar* = 50 μm. All light micrographs are at the same scale. Leaves were sampled during the wet season.

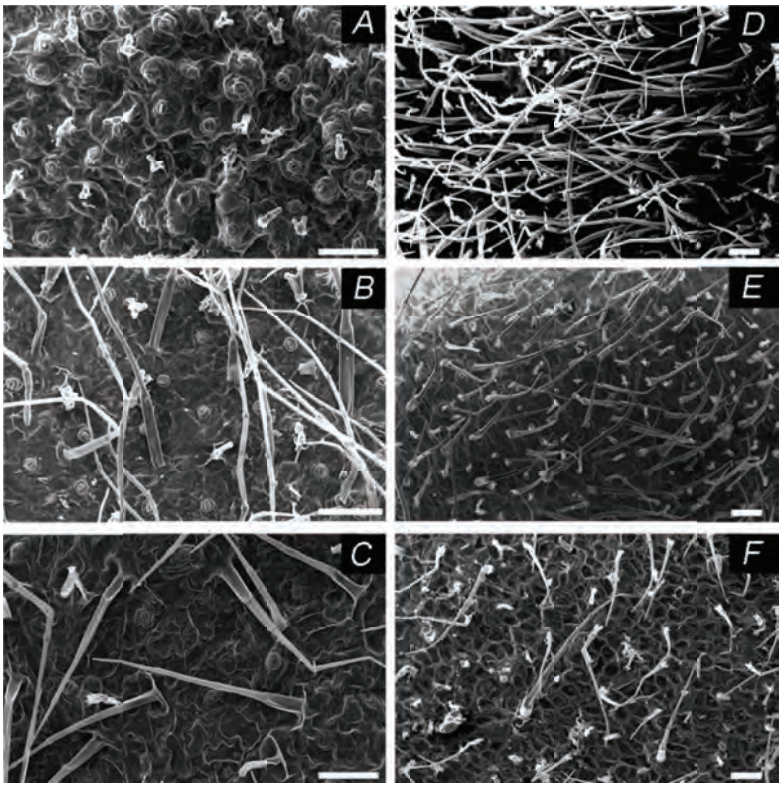

Fig. 3. Density of stomata and epidermal hairs in leaves grown at three sites. Stomatal density of leaves from the open area (OA) site (*A*), the entrance zone (EZ) site (*B*), and the twilight zone (TZ) site (*C*). Epidermal hair density of leaves from the OA site (*D*), the EZ site (*E*), and the TZ site (*F*). Micrographs are at 200 × magnification for *A*–*C* and 100 × magnification for *D*-*F*. *Bars* = 100 μm.

among sites was EZ>OA>TZ (Table 6). The Car content per unit leaf area, the ratio of Chl *a/b*, and the ratio of Car/Chl increased with increase in light availability so that the order among the sites was OA>EZ>TZ. Lower ratios of Chl *a/b* and Car/Chl at the cave ecotone sites than at the open site are in accordance with the characteristics of shade plants. An extremely low Chl content was found in leaves at the TZ site (about 46-60% of that at the EZ site).

Morphological characteristics, anatomy, and chloroplast ultrastructure of *P. tabacum*: Total biomass and biomass allocation of *P. tabacum* differed ($p<0.05$) among the three sites (Table 6). The biomass was 55% lower at TZ site than at the EZ and OA sites. The belowground/aboveground biomass ratio was significantly smaller at the cave ecotone sites than at the OA site, LMA was significantly higher at the OA and EZ sites than at the TZ site, whereas the leaf area ratio (LAR) and leaf mass fraction (LMF) were highest at the TZ site and lowest at the OA site.

The anatomy of *P. tabacum* also significantly differed among the three sites (Table 6). Stem diameter and stem vascular density were highest at the OA site, intermediate at the EZ site, and lowest at the TZ site. As evidenced by light micrographs of leaf transverse sections (Fig. 2) and SEM micrographs of leaf surfaces (Fig. 3), the pubescence density on the leaf surface, the leaf thickness and stomata density, as well as the thickness of palisade and sponge tissue (also shown in Table 6) were correlated with light intensity of the sites. At the OA site, the leaf mesophyll was clearly differentiated into palisade and spongy layers, and leaf thickness was about 15 and 55% greater than at the EZ site and TZ site, respectively. The

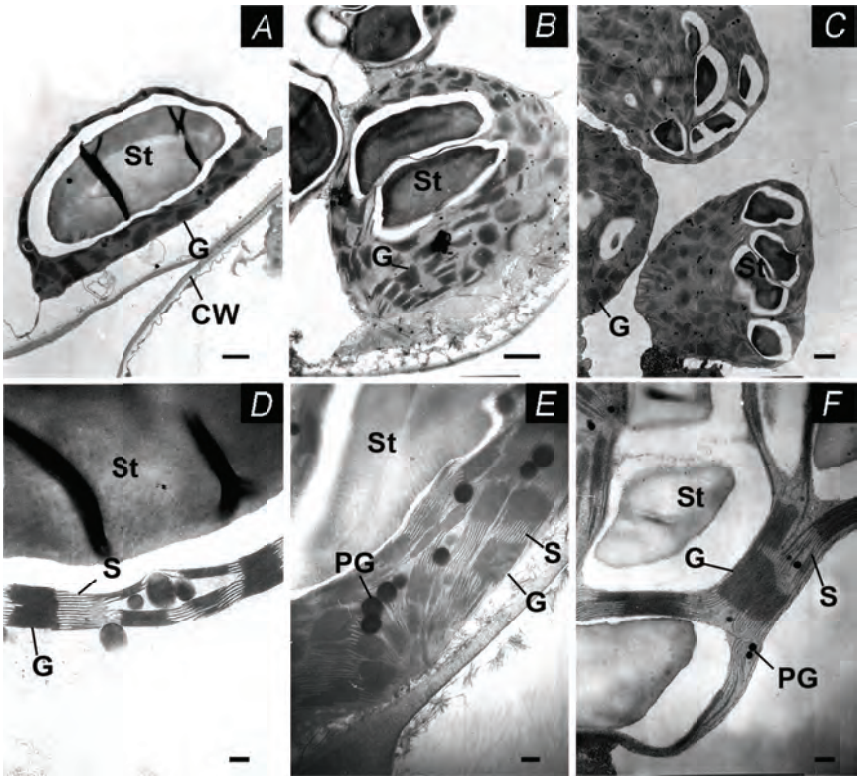

Fig. 4. Ultrastructure of leaf chloroplasts and grana lamella from three sites. Leaf chloroplasts from the open area (OA) (*A*), the entrance zone (EZ) (*B*), and the twilight zone (TZ) (*C*). Grana lamella from OA (*D*), EZ (*E*), and TZ (*F*). Note that chloroplasts contain fewer grana and larger starch granules in leaves from OA than from EZ or TZ. CW – cell wall; St – starch granule; G – grana lamella; S – stroma lamella; PG – plastoglobule. *Bar* = 1 μm for *A–C* and 200 nm for *D–F*.

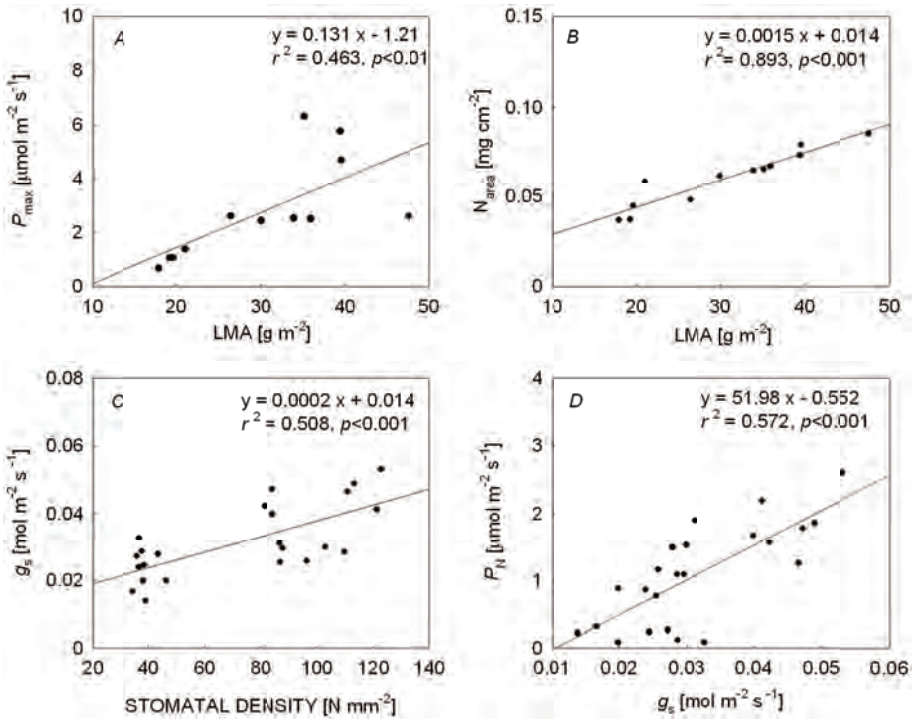

Fig. 5. The relationship between P_{max} and LMA, N_{area} and LMA, g_s and stomatal density, and P_N and g_s in leaves of *P. tabacum*.

greater leaf thickness at the OA site was accompanied by an increase in both spongy and palisade mesophyll thickness and a higher palisade/spongy ratio, indicating that the increment in leaf thickness was primarily due to the increase in palisade thickness. At the EZ and TZ sites, in contrast, the palisade and spongy parenchyma were not clearly differentiated, the palisade parenchyma contained large intercellular spaces and the cells of the palisade

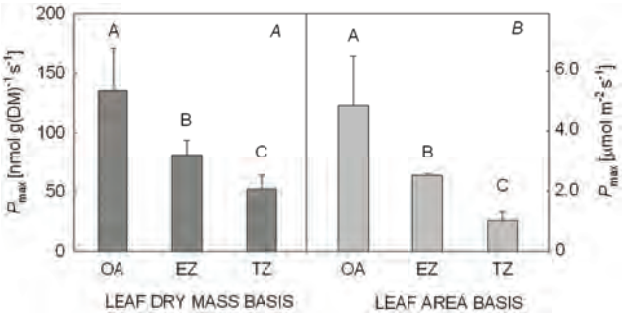

Fig. 6. Maximal net photosynthetic rate (P_{max}) expressed on leaf dry mass basis (*A*), and on leaf area basis (*B*). Values are means (± SD, n = 5). Within each panel, bars with *different letters* are significantly different at $p<0.05$.

parenchyma did not conform to the typical rectangular shape.

The ultrastructure of *P. tabacum* chloroplasts also differed among the sites (Table 6, Fig. 4). Chloroplasts in leaves at the OA site had a low number of grana per chloroplast (23 and 26% lower than at the EZ and TZ sites), reduced stacking of thylakoids (12 and 28% lower than at the EZ and TZ sites), and large starch grains. Clearly, high light in OA site reduced the quantity of grana per chloroplast and thylakoid per granum but increased the accumulation of starch grains. In contrast, chloroplasts in leaves receiving faint light at the cave ecotone sites had well-developed grana and a densely stacked thylakoid system.

Relationships among some morphological and physiological properties: Regression analyses indicated that LMA was positively related to P_{max} and N content on a leaf area basis (Fig. 5*A*,*B*). The correlation coefficients (r) were 0.680 (for P_{max}) and 0.945 (for N content), indicating that LMA was one of the main morphological factors associated with the differences in P_{max}, N content, and light intensity. In addition, stomata density and g_s was positively related to P_N (Fig. 5*C*,*D*), indicating that the stomata density on the leaf surface could also be important for *P. tabacum* photosynthesis. Similar trends were observed in P_{max} on dry mass basis and on leaf area basis (Fig. 6). With P_{max} expressed on leaf area unit, *P. tabacum* exhibited significantly lower P_{max} in leaves at EZ (48%) and TZ (78%) sites than at OA site (Fig. 6*A*). Even on leaf dry mass basis the P_{max} per leaf mass unit was significantly lower at EZ (40%) and TZ (61%) sites than at OA site (Fig. 6*B*).

Discussion

N/P ratio in plant tissue lower than 14 is considered to be indicative of N deficiency (Koerselman and Meuleman 1996, Güsewell and Koerselman 2002, Tessier and Raynal 2003). The low N/P ratio in the aboveground biomass of *P. tabacum* suggests that N rather than P was the most limiting nutrient for *P. tabacum* growing in the karst region.

The three sites used in this study were very similar in terms of nutrient availability but very different in terms of light intensity, CO_2 concentration, and moisture availability. Therefore, *P. tabacum* growing in these different habitats has been subjected to a long period of different selective pressures, and our results indicate that the different light conditions oriented habitats impart significant differences in the structural and physiological characteristics of *P. tabacum* adaptations. As discussed in the following two paragraphs, the OA population exhibited traits characteristic of sun-adapted and drought-tolerant plants whereas the populations growing at cave vicinity exhibited characteristics of shade-tolerant plants.

P. tabacum growing in the open area (site OA) differed from *P. tabacum* growing in the vicinity of the cave in many aspects. The OA population experienced high light intensity and seasonal drought due to the specified geological and climatic characteristics of karst areas, and they tended to have large belowground stems with a dense vascular system, thick leaves with high LMA, high stomata density and pubescence density, well-developed palisade parenchyma, as well as chloroplasts with a sun-type ultrastructure. Increased palisade thickness is an adaptation to drought or high irradiance (Lichtenthaler 1985, Guerfel 2009) and can result in an increased photosynthetic capacity of the leaf (Sefton *et al.* 2002). The dense pubescence on the leaf surface of the OA population would prevent or reduce photodamage; the pubescence would reflect and scatter the light, resulting in considerable shading of the chlorenchyma, and could also increase drought resistance by reducing transpiration (Croteau 1977, Werker 1993, Kolb and Müller 2004). The larger belowground stem and dense vascular system might be helpful for water storage and uptake under drought conditions. Meanwhile, the physiological characteristics of the OA population were also associated with high tolerance to high irradiance and drought stress. The higher P_{max}, LCP, and LSP in the OA population than in the cave sites oriented populations indicate that the OA population could effectively utilize higher light than the site of cave vicinity populations. Analysis of chlorophyll fluorescence confirmed the differences in photosynthetic capacity between the OA and cave sites oriented populations. For the OA population, the elevated capacity of electron transport (higher $rETR_{max}$) under natural irradiance would enhance RuBP regeneration and the activities of photosynthetic enzymes (Harbinson *et al.* 1989), which would subsequently result in a high photosynthetic capacity. NPQ is a protective mechanism related to the xanthophyll cycle pigment pool (component of Car), which protects plants from photooxidative damage by increasing thermal energy dissipation in the PSII antennae (Demmig-Adams

et al. 1996, Horton *et al*. 2005). In this study, the high NPQ accompanied with higher carotenoid content and Car/Chl ratio in the OA population indicated that *P. tabacum* had an enhanced capacity to dissipate the excess excitation energy resulting from high light intensity as heat.

In caves, light is the major factor that limits growth and distribution of the plants. Low irradiance deprives plants of their major source of energy and reduce the production of biomass, and thus the selective pressures in cave vicinity habitat should favour plants with traits for optimization of light capture. Leaf morphology, anatomy, chloroplast ultrastructure, and physiological activity are all dependent on the prevailing light condition. Those anatomical traits of *P. tabacum* at the cave oriented sites that would increase growth and survival under low light were a significantly reduced LMA and a reduced palisade-to-spongy parenchyma ratio. A decreased LMA can improve light harvesting per unit of resource invested in the construction of photosynthetic tissues (Lusk *et al*. 2008), and the lower palisade-to-spongy parenchyma ratio scatters irradiance internally, thus increasing light absorption by the leaf (Lawren *et al*. 2006, Matos *et al*. 2009). On the other hand, the higher aboveground/ belowground biomass ratio and LMF in cave oriented site indicate that more biomass was allocated to the light-capturing organs for developing a larger light-absorbing surface in irradiance-limited environments. Those physiological traits of *P. tabacum* at the cave vicinity sites that would increase growth and survival under low light were decreases in the Chl *a/b* ratio, LSP, LCP, and R_D of leaves, and decreases in Φ_{PSII}, q_P, and NPQ of photosystem II. The lower Chl *a/b* ratio and higher degree of stacking of thylakoids are generally considered to be indicative of a larger proportion of Chl *a/b*-binding light-harvesting complexes (LHC) – an adaptation that is often regarded as one that maximizes light capture in low light (Barber 1980, Anderson *et al*. 2008). The lower R_D and LCP could enable *P. tabacum* to decrease respiratory carbon losses and to maintain a positive carbon balance at lower light levels in caves (Man and Lieffers 1997, Craine and Reich 2005).

The anatomical structure of leaf and regression analyses between LMA and P_{max} indicated that P_N of *P. tabacum* was significantly influenced by leaf thickness. As compared to thinner shade leaves, thicker leaves in sun habitats contain significantly more cells and chloroplasts per leaf area unit for photosynthesis (Lichtenthaler 1981, 1985). However the net photosynthesis of leaf depends not only on the mesophyll structure but also on the photosynthetic biochemistry of leaves. According to Kubiske and Pregitzer (1997), shade leaves of shade-intolerant species respond to shade primarily by altering SLA, whereas shade-tolerant species respond in large part *via* biochemical acclimation of the photosynthetic apparatus. In this study we found that the leaves in shade cave habitats had significantly lower leaf-area-based P_{max} with a large decrease in dry-mass-based P_{max}. A similar result was also reported by Lichtenthaler *et al*. (2007) in other shade-tolerant species (*Abies alba* and *Tilia cordata*), indicated that *P. tabacum* responded to shade largely *via* biochemical acclimation of the photosynthetic apparatus.

All of these findings indicate that *P. tabacum* populations adapted in contrast habitats differ remarkably in structure and physiology traits, and such variabilities are important for survival of *P. tabacum* in heterogeneous light conditions. According to Tobin and Silverthore (1985), light is the most important factor regulating gene expression in higher plants. The expression of many genes including those that encode mRNA of Rubisco LSU and Chl *a/b* apoprotein are altered by light, and chloroplast development is related to the expression of genes for both chloroplast- and nuclear-encoded proteins. Recent research on genetic diversity revealed high levels of genetic differentiation among *P. tabacum* populations, possibly resulting from the restricted gene flow due to isolation of populations by geographical barriers (Ni *et al*. 2006, Wang *et al*. unpublished data). Thus, in the current study the observed divergence between the OA population and the cave oriented populations could be explained by both genetic differentiation and phenotypic plasticity. It is possible that there is a genetic or ecotypic differentiation among the populations of *P. tabacum*, which adapted in different habitats with contrasting selective pressures. However, whether such divergence is the outcome of phenotypic plasticity or a consequence of genetic differences among populations is a question worthy of further research.

Previous experimental studies have reported higher P_{max}, V_{cmax}, and lower g_s under elevated CO_2 as compared to low CO_2 conditions (Fernández *et al*. 1999, Košvancová *et al*. 2009). These results are in accordance with our findings. In EZ and TZ sites, higher P_{max} and V_{cmax} were accompanied with higher C_a and C_i when comparing wet and dry season. In addition, the significantly higher C_a values and lower g_s were found in cave sites in wet season. It implies that the elevated CO_2 in cave would probably have an unnegligible effect on the gas exchange and CO_2 assimilation of *P. tabacum* in wet season. Generally, the increase in CO_2 assimilation rate as a result of CO_2 elevation was often found in C_3 plants (Ceulemans and Mousseau 1994, Raines 2006). Since increased CO_2 concentration results in higher photosynthesis under high-light condition through a faster photoactivation of Rubisco (Košvancová *et al*. 2009), suppression of photorespiration and increased substrate level for photosynthesis (Dijkstra *et al*. 1999), here in the cave we suppose that the elevated CO_2 in wet season may play a compensatory role in increasing CO_2 assimilation under light-limited habitat.

Finally, as compared to open area, the cave ecotone is usually maintained at lower irradiation and higher humidity inside, *P. tabacum* can avoid injury caused by

strong irradiation and water stress in this 'shelter'. Thus we suggest preventive measures should be taken to protect this species and its cave habitats from anthropogenic exploitation and destruction.

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