

## BRIEF COMMUNICATION

## Photosynthetic characteristics involved in adaptability to Karst soil and alien invasion of paper mulberry (*Broussonetia papyrifera* (L.) Vent.) in comparison with mulberry (*Morus alba* L.)

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### Abstract

Unlike mulberry (*Morus alba*, *M.a.*), paper mulberry (*Broussonetia papyrifera*, *B.p.*) can acclimate to Karst soil and incline to alien invasion. The photosynthetic parameters, diurnal changes of carbonic anhydrase, and chlorophyll fluorescence induction, and water potential were measured on sunny days (SD) and cloudy days (CD). Photosynthetic midday depression occurred in *B.p.* but not in *M.a.* The irradiance- and CO<sub>2</sub>-saturated photosynthetic rates of *B.p.* were significantly higher than those of *M.a.* There was no significant difference in water use efficiency between the two species on a SD. The maximum fluorescence, maximum quantum yield, photochemical quenching, and relative electron transport rate in the leaves of *B.p.* were much higher than those in *M.a.* The activity of carbonic anhydrase (CA) of *B.p.*, on either an SD or a CD, was much greater than that of *M.a.* Higher transpiration rate (*E*) and net photosynthetic rate (*P<sub>N</sub>*) of *B.p.* resulted in the lack of water in mesophyll cells. Although a higher CA activity of *B.p.* supplied both water and CO<sub>2</sub> for the photosynthesis of mesophyll cells, water in mesophyll cells was the factor limiting photosynthesis, and the intercellular CO<sub>2</sub> concentration of *B.p.* was high and stable.

*Additional key words:* carbonic anhydrase; chlorophyll *a* fluorescence; mulberry; photochemical and non-photochemical quenching; species differences; stomatal conductance; transpiration rate; water potential.

Paper mulberry (*Broussonetia papyrifera* (L.)Vent.; *B.p.*) and mulberry (*Morus alba* L.; *M.a.*), perennial tree species belonging to Moraceae, are characterized by higher growth rate and greater adaptability to adverse environments than other species in this family (Zhang *et al.* 1998, Zhao *et al.* 2005). They have been cultivated extensively in East, Central, and South Asia for papermaking, silk and timber production, and medical materials (Qin *et al.* 1999, Ouyang and Chen 2003).

Many investigations have addressed such physiological aspects as changes of peroxidase, esterase isozyme activities, and endogenous indole-3-acetic acid during cambial activity, the regeneration from leaf mesophyll protoplasts, and the accumulation of dry matter of each

organ and various parts in paper mulberry (Oka and Ohyama 1985, Cui *et al.* 1995a,b, 1999, Dai 1995). Karst areas often suffer from water stress because of the unique geo-environment (Zhou *et al.* 2003). Therefore, drought tolerance is vital to the plants in the Karst area. Recent studies have also shown that this plant species, which is native to East Asia, has become an invader on several continents and in more than dozen countries (Ghersa *et al.* 2002, Morgan and Overholt 2005).

Certain physiological changes of mulberry in response to water stress, high temperature, and toxic effects of heavy metals have been studied (Ramanjulu *et al.* 1998a,b, Kumar *et al.* 1999, Barathi *et al.* 2001, Chaitanya *et al.* 2001, 2003, Thimmanaik *et al.* 2002, Wang *et al.* 2004).

Received 23 July 2008, accepted 7 October 2008.

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*Acknowledgments:* We gratefully acknowledge the financial support from National Basic Research Program of China (973 Program) (No. 2006CB403206) and projects of National Natural Science Foundation of China (No. 40273038). We are grateful to anonymous reviewers for valuable comments and suggestions on the manuscript.

Mulberry trees of different species and origins adapt differently to adverse conditions, which results in the regional distribution of the trees (Tateno and Taneda 2007). But the mulberry is not the only pioneer plant species in Karst areas, and there is no record that this plant is an invasive species.

Photosynthetic activities can be represented as the growth potentials of the plant (Mooney 1972, Walters *et al.* 1993, McAllister *et al.* 1998, Knee and Thomas 2002). Chlorophyll (Chl) *a* fluorescence of photosystem 2 plays an important role in the response of leaf photosynthesis to environmental stresses (Baker 1991). The increasing photosynthetic energy-use efficiency and enhancing photosynthetic capacity may be the most successful mechanism for alien species invasion, and for adaptability to adverse environment (Nagel and Griffin 2004, Zhang *et al.* 2006). Thus, the two species might differ from each other in their photosynthetic characteristics involved in adaptability to Karst environment and alien invasion.

Carbonic anhydrase (CA, EC 4.2.1.1), a zinc-containing metalloenzyme that catalyses the reversible conversion of CO<sub>2</sub> to bicarbonate, is widely distributed in animals, plants, archaea, and eubacteria, where it is involved in diverse physiological processes, such as ion exchange, acid-base balance, carboxylation/decarboxylation reactions, and inorganic carbon diffusion between the cell and its environment as well as within the cell (Badger and Price 1994, Sasaki *et al.* 1998). CA regulates photosynthesis in response to water stresses.

The present study aims to understand their fast-growing and adaptability to water stress, and examine the *B.p.* invasive characteristics by comparing its photosynthetic characteristics and water-use efficiency (WUE) with those of *M.a.* The experiment was conducted from 1<sup>st</sup> October 2006 to 30<sup>th</sup> November 2006 on a plantation in suburban Guiyang, Southwest China. Forty actively growing, 3-year-old and about 2–3 m tall *B.p.* and *M.a.* were used. The sixth leaf from the top of branches (recently matured leaf) was used for the measurement of the net photosynthetic rate ( $P_N$ ), leaf water potential ( $\Psi_L$ ), CA activity, and Chl *a* fluorescence. The diurnal course of  $P_N$  and its response to irradiance and internal CO<sub>2</sub> concentration ( $C_i$ ) were measured on the attached leaves using a portable measurement system *LI-6400* (*LI-COR*, Lincoln, NE, USA) during October 2006. The diurnal changes of  $P_N$  on three typical sunny (SD) and three typical cloudy (CD) days were measured from 08:00 to 17:00. Five replicate measurements of the two species were made on both SD and CD. In addition, air temperature ( $T_a$ ), photosynthetic photon flux density (PPFD), transpiration rate ( $E$ ), and stomatal conductance ( $g_s$ ) were determined. WUE was calculated by dividing  $P_N$  by  $g_s$ . Irradiation was supplied with *6400-02B* red-blue light emitting diodes (*LI-COR*). Irradiances used for the construction of curves were 2 000, 1 800, 1 500, 1 200, 1 000, 800, 600, 400, 300, 200, 150, 100, 50, and 0  $\mu\text{mol}$

$\text{m}^{-2} \text{s}^{-1}$ . Leaf cuvette reference CO<sub>2</sub> concentration was 400  $\mu\text{mol mol}^{-1}$ , and temperature was 18.0±0.5 °C. Irradiance and CO<sub>2</sub> responses of  $P_N$  were modelled with non-rectangular hyperbolae. Chl *a* fluorescence was measured with a *MINI-PAM* light-modulated fluorimeter (Walz, Germany) at room temperature (25±2 °C). The leaves in the experiments were adapted for darkness for 30 min to guarantee the oxidised state of quinone A. The fluorescence parameters assessed were as follows: initial fluorescence ( $F_0$ ) obtained with modulated low irradiance (<0.1  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), maximum fluorescence ( $F_m$ ) determined with a pulse of saturating irradiance (~6 000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) with duration of 0.3 s, and maximum quantum yield of photosystem 2 (PS2):  $F_v/F_m = (F_m - F_0)/F_m$ . Hence, these parameters were used to obtain the photochemical quenching:  $q_p = (F'_m - F)/(F'_m - F_0)$ , and the non-photochemical quenching:  $q_N = (F_m - F'_m)/(F_m - F_0)$ . These quenching coefficients were automatically calculated with the *MINI-PAM*. Electron transport rate was estimated as  $\text{ETR} = \Delta F/F'_m \times \text{PAR} \times 0.5 \times 0.84$ , where  $\Delta F/F'_m$  is the effective quantum yield of irradiated sample (variable fluorescence/effective maximum fluorescence), and PAR is photosynthetically active radiation (Ferreira *et al.* 2007).  $q_p$ ,  $q_N$ , and ETR were measured in response to different PAR consisting of 11 consecutive periods of “actinic light” (0, 41, 76, 134, 205, 249, 298, 371, 456, 581, and 726  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). Each photosynthetic photon flux (PPF) period of “actinic light” lasted about 20 s following a pulse of saturating radiation (6 000  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) of 0.3 s duration. Before the sequence of measurements with increasing “actinic light”, one measurement was performed in the dark.

$\Psi_L$  of attached leaves was measured with a Dew Point Hygrometer *HR-33T* with leaf chamber *L-51* (*Wescor*, Logan, USA). The leaves had been kept in the chamber for 2–4 min before  $\Psi_L$  was estimated. Diurnal patterns of leaf water potential were also measured from 08:00 to 17:00. Five replicate measurements of the two species were taken in both SD and CD.

The activities of CA were determined using the pH method (Wu *et al.* 2005). The leaves of 0.5 g were quickly frozen in liquid N<sub>2</sub>, ground into powder with a mill, then homogenized with 3 cm<sup>3</sup> of extraction buffer (10 mM veronal buffer with 50 mM 2-mercaptoethanol, pH 8.2). The homogenate was centrifuged at 10 000×*g* for 5 min. CA activity in the above 0.2–1.0 cm<sup>3</sup> supernatants was electrochemically determined by measuring the time required for the pH to drop from 8.2 to 7.2 in 15 cm<sup>3</sup> of ice-cold barbital buffer (20 mM, pH 8.30) with 10 cm<sup>3</sup> of ice-cold CO<sub>2</sub>-saturated distilled H<sub>2</sub>O. One WA unit (Wilbur and Anderson 1948) of activity was defined as:  $\text{WA} = t_0/t - 1$ , where  $t_0$  and  $t$  are the time required for the pH to drop one unit, respectively, in the enzyme-free buffer (control) and the buffer containing enzyme sample. The diurnal patterns of leaf CA activity were also measured from 08:00 to 17:00 for the SD and CD. Five replicate measurements of the two species were made

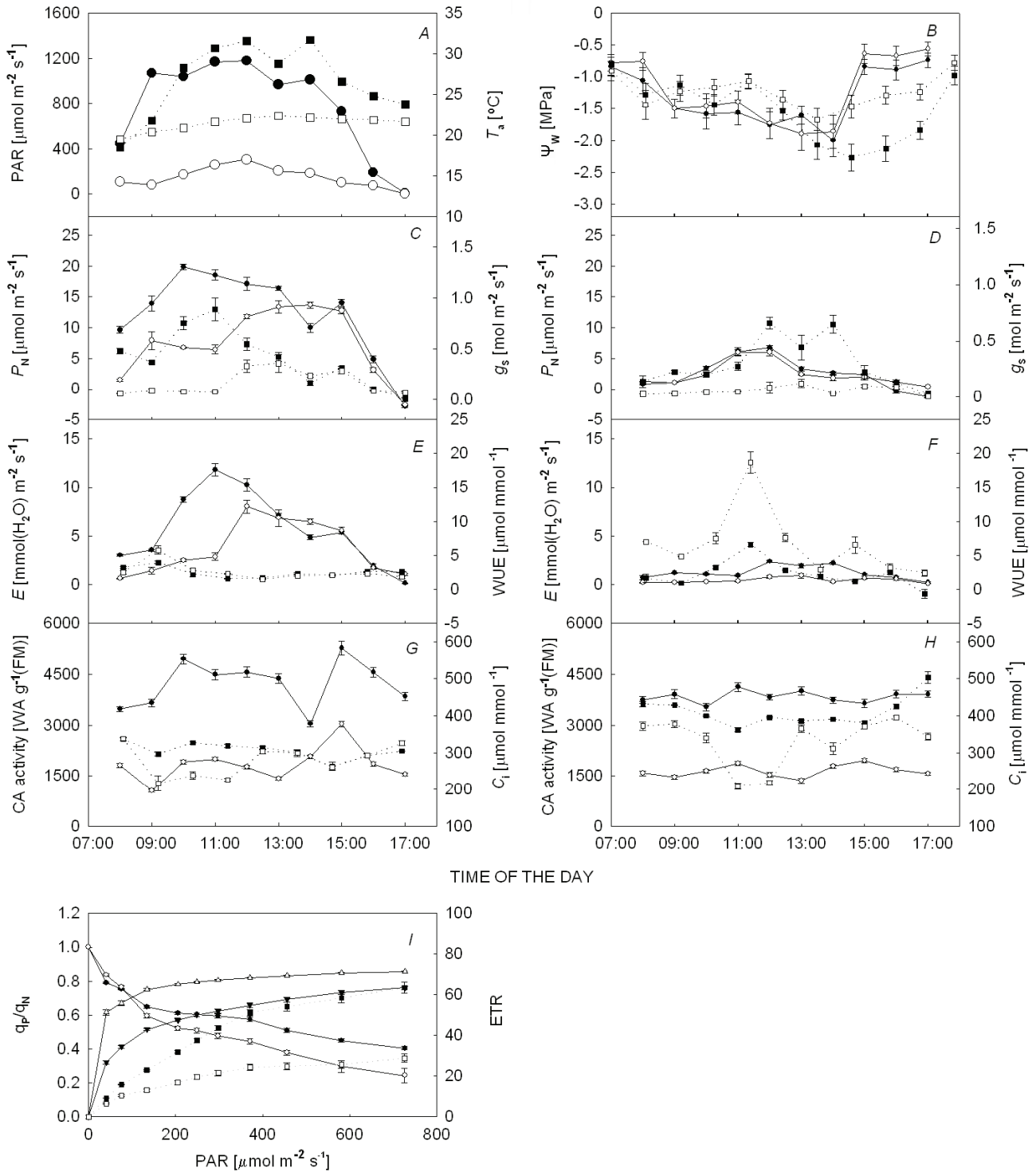


Fig. 1. Diurnal patterns of (A) photosynthetically active radiation, PAR ( $\bullet$  sunny day, SD;  $\circ$  cloudy day, CD) and air temperature,  $T_a$  ( $\blacksquare$  SD;  $\square$  CD); (C, D) net photosynthetic rate,  $P_N$  ( $\bullet$  paper mulberry;  $\circ$  mulberry) and stomatal conductance,  $g_s$  ( $\blacksquare$  paper mulberry;  $\square$  mulberry) (C SD; D CD), (E) transpiration rate,  $E$  ( $\bullet$  paper mulberry;  $\circ$  mulberry) and water use efficiency, WUE ( $\blacksquare$  paper mulberry;  $\square$  mulberry) (E SD; F CD), (G, H) carbonic anhydrase activity, CA ( $\bullet$  paper mulberry;  $\circ$  mulberry) and internal  $\text{CO}_2$  concentration,  $C_i$  ( $\blacksquare$  paper mulberry;  $\square$  mulberry) (G SD; H CD), and (B)  $\Psi_w$  ( $\bullet$  paper mulberry,  $\circ$  mulberry on SD;  $\blacksquare$  paper mulberry,  $\square$  mulberry on CD) during the SD (9 October 2006) and the CD (12 October 2006). (Means  $\pm$  SE,  $n = 5$ ). (I) Photochemical,  $q_p$  ( $\bullet$  paper mulberry;  $\circ$  mulberry) and non-photochemical quenching,  $q_N$  ( $\blacktriangledown$  paper mulberry;  $\triangle$  mulberry), and relative electron transport rate, ETR ( $\blacksquare$  paper mulberry;  $\square$  mulberry) in response (Means  $\pm$  SE,  $n = 10$ ) to PAR in the leaves of in paper mulberry and mulberry.

Table 1. Irradiance-saturated rate of net photosynthesis ( $P_{\text{sat}}$ ) [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ], the  $\text{CO}_2$ -saturated rate of net photosynthesis ( $P_{\text{max}}$ ) [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ], compensation irradiance (CI) [ $\mu\text{mol m}^{-2} \text{s}^{-1}$ ],  $\text{CO}_2$  compensation concentration ( $\Gamma$ ) [ $\mu\text{mol mol}^{-1}$ ], quantum efficiency ( $\epsilon$ ), carboxylation efficiency (CE) [ $\text{mol m}^{-2} \text{s}^{-1}$ ] (Means  $\pm$  SE,  $n = 5$ ), initial ( $F_0$ ) and maximum fluorescence ( $F_m$ ), and maximum quantum yield ( $F_v/F_m$ ) (Means  $\pm$  SE,  $n = 10$ ) in paper mulberry (*B.p.*) and mulberry (*M.a.*). The different letters following the figures in the same columns represent significant difference ( $p < 0.05$ ) between the two species.

Plant	$P_{\text{sat}}$	$P_{\text{max}}$	CI	$\Gamma$	$\epsilon$	CE	$F_0$	$F_m$	$F_v/F_m$
<i>B.p.</i>	10.94 $\pm$ 0.28b	25.96 $\pm$ 3.39b	31.91 $\pm$ 2.12b	59.68 $\pm$ 2.75b	0.073 $\pm$ 0.009a	0.013 $\pm$ 0.001a	0.117 $\pm$ 0.005a	0.441 $\pm$ 0.012b	0.736 $\pm$ 0.005b
<i>M.a.</i>	5.21 $\pm$ 0.21a	13.72 $\pm$ 0.73a	17.84 $\pm$ 1.56a	38.35 $\pm$ 2.01a	0.072 $\pm$ 0.015a	0.050 $\pm$ 0.009b	0.135 $\pm$ 0.006a	0.321 $\pm$ 0.011a	0.578 $\pm$ 0.018a

during both SD and CD.

Statistical analyses were carried out with SPSS version 9.0 (SPSS, Chicago, IL, USA). The differences between the two species were evaluated by one-way analysis of variance (ANOVA) or Student's *t*-test.

The PAR and air temperature ( $T_a$ ) on a SD (9 October 2006) and a CD after a heavy rain (12 October 2006) changed as shown in Fig. 1A. On the SD, PAR and  $T_a$  peaked at the midday. On the CD, PAR was relatively low, and the change of  $T_a$  within one day was not significant. The diurnal curves of  $P_N$  and  $g_s$  on the SD (Fig. 1C) showed that the  $P_N$  and  $g_s$  in *B.p.* were much higher than those in *M.a.* from 08:00 to 13:00. The peak  $P_N$  and  $g_s$  in *B.p.* appeared at 10:00 and 11:00, respectively, and those in *M.a.* at 13:00. After 15:00, there were no significant differences between *B.p.* and *M.a.* "Photosynthetic midday depression" appeared in *B.p.*, but not in *M.a.* On CD (Fig. 1D) the peak values of  $P_N$  in *B.p.* and *M.a.* appeared at 12:00, and  $P_N$  in the *B.p.* was not different from that in *M.a.* The  $g_s$  in *B.p.* was much higher than that in *M.a.*

*B.p.* had higher  $E$  than *M.a.* on SD or CD (Fig. 1E,F). WUE of *B.p.* on CD was lower than that of *M.a.* WUE was similar in the two species on the SD except at 09:00.

On both SD and CD (Fig. 1G,H), the CA activity in *B.p.* was higher than that in *M.a.* The changes of CA activity on SD were significant, whereas those during the CD were small. The lowest CA activity in *B.p.* occurred at 14:00 in the afternoon on the SD, and peaked at around 15:00.

The  $C_i$  of *B.p.* changed within narrow ranges. During SD, the  $C_i$  in *B.p.* from 09:00 to 11:00 was much higher than that in *M.a.* (Fig. 1G). On the CD, the  $C_i$  in *B.p.* was higher than that in *M.a.* within a day (Fig. 1H).

The diurnal course of  $\Psi_w$  in *B.p.* on SD (Fig. 1B) remained at a similar tendency to that in *M.a.* The lowest value occurred at 14:00, and during the CD the  $\Psi_w$  in *B.p.* was significantly lower than that in *M.a.* (Fig. 1B).

Irradiance ( $P_{\text{sat}}$ ) and  $\text{CO}_2$ -saturated net photosynthesis ( $P_{\text{max}}$ ) in *B.p.* were significantly higher than those *M.a.* and compensation irradiance (CI) and compensation  $\text{CO}_2$  concentration ( $\Gamma$ ) in *B.p.* were significantly higher than those in *M.a.* The carboxylation efficiency (CE) in *B.p.* was significantly lower than that in *M.a.* while  $P_{\text{max}}$  was significantly higher than that in *M.a.* (Table 1). However, significant difference was not observed in quantum

efficiency between *B.p.* and *M.a.*

No significant difference was observed for  $F_0$  in the leaves of *B.p.* and *M.a.* (Table 1). The  $F_m$  and  $F_v/F_m$  in the leaves of *B.p.* were much higher than those in *M.a.* Hence *B.p.* had a higher photochemical efficiency of PS2 than *M.a.* The  $q_p$  in *B.p.* was almost higher than that in *M.a.* except under weak irradiance ( $< 134 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), and  $q_N$  in *B.p.* was significantly lower than that in *M.a.* (Fig. 1I). This suggests that the *B.p.* has a greater capacity to regenerate the photosynthetic apparatus. The *B.p.* had also a higher relative ETR than *M.a.*

The *M.a.* is one of the plants with high drought tolerance due to its high WUE (Ramanjulu *et al.* 1998a,b). But we found less difference between *B.p.* and *M.a.* in WUE, even at noon of a SD. This suggests that *B.p.* and *M.a.* have a similar drought tolerance.

Karst areas often suffer from soil water-logging after a heavy rain (Lin and Zhu 1999), and many plants cannot grow well and gradually die of root rotting during soil water-logging. Higher  $E$  and lower WUE in *B.p.* on CD can cause more water to evaporate into the atmosphere, and reduce the water contents of soil. Thus the adaptability of *B.p.* to soil water-logging is better than that of *M.a.*

Although higher  $E$  and  $P_N$  of *B.p.* on SD resulted in the lack of water in mesophyll cells and  $C_i$  and CA may provide both  $\text{H}_2\text{O}$  and  $\text{CO}_2$  for the photosynthesis of mesophyll cells, *i.e.* higher CA activity may be a contributor to the higher  $C_i$  and more water for the photosynthesis of mesophyll cells. Therefore,  $P_N$  in *B.p.* was higher than that in *M.a.* until 12:00. The activity of CA in *M.a.* was also very high compared with that in some other plants (Wu *et al.* 2006), which may explain why no photosynthetic midday depression similar to that in *Orychophragmus violaceus* was observed (Wu *et al.* 2005). But, the activity of CA in *B.p.* was about 2 times higher than that in *M.a.* Why did the photosynthetic midday depression occur in *B.p.* with high CA activity? It was because CA transforms  $\text{HCO}_3^-$  into  $\text{H}_2\text{O}$  and  $\text{CO}_2$  in the rate of 1 : 1 even though the CA activity of *B.p.* was higher than that of *M.a.* The  $P_N$  and  $E$  in *B.p.* were significantly higher than those in *M.a.* in the midday, and the more water was lost, the more  $\text{CO}_2$  was held in the mesophyll cells. Water in mesophyll cells of *B.p.* became a factor limiting photosynthesis during high temperature and low humidity, and the  $C_i$  of *B.p.* was high and stable. Moreover, the CA activity in *B.p.* was at the lowest

at 14:00, and at the highest at 15:00 on the SD, *i.e.* no at 14:00.

Many studies have shown that the invasive species have significantly higher photosynthetic capacity than the non-invasive species (McDowell 2002, Nagel and Griffin 2004, Zhang *et al.* 2006). The fact that *B.p.* had higher  $F_m$ ,  $F_v/F_m$ ,  $q_p$ , ETR, and lower  $q_N$  than *M.a.* indicates the

sufficient CA activity regulated the water stress in *B.p.* superior capacity and the higher efficiency of energy capture of *B.p.* (McDowell 2002, Castillo *et al.* 2007). The lower value of  $F_v/F_m$  in both cases may be caused by the „karstic drought“. Higher  $P_{max}$  in *B.p.* also indicates that it has stronger invasive capacity than *M.a.*

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