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Effect of drought on pigments, osmotic adjustment and antioxidant enzymes in six woody plant species in karst habitats of southwestern China

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ABSTRACT

Drought stress is one of the most important factors limiting the survival and growth of plants in the harsh karst habitats of southwestern China. Detailed knowledge about the ecophysiological responses of native plants with different growth forms to drought stress could contribute to the success of re-vegetation programs. Two shrubs, *Pyracantha fortuneana* and *Rosa cymosa*, and four trees, *Broussonetia papyrifera*, *Cinnamomum bodinieri*, *Platycarya longipes* and *Pteroceltis tatarinowii*, were randomly assigned to four drought treatments, i.e. well-watered, mild drought stress, moderate drought stress, and severe drought stress. Midday water potential, the maximum quantum efficiency of PSII photochemistry (F_v/F_m), pigments, osmotic solutes (soluble sugars and proline), cellular damages, and antioxidant enzymes (superoxide dismutase, catalase and peroxidase) were investigated. Drought stress significantly decreased pigments content, but increased the ratio of carotenoids to total chlorophylls in the studied species. After prolonged severe drought stress, the two shrubs exhibited higher F_v/F_m, less reductions of midday water potential, and lower increases of malondialdehyde content and ion leakage than the four trees. Prolonged severe drought stress largely decreased accumulations of osmotic solutes and activities of antioxidant enzymes in the four trees, but significantly increased proline content and superoxide dismutase activity in the two shrubs and peroxidase activity in *P. fortuneana*. The positive relationships were observed among activities of antioxidant enzymes, and between contents of osmotic solutes and activities of antioxidant enzymes. These findings suggested that the two shrubs had higher tolerance to severe drought stress than the four trees due to higher capacities of osmotic adjustment and antioxidant protection.

1. Introduction

The karst landscape in southwestern China is one of the most typical landscapes developed on limestone in the world (Liu, 2009). Karst habitats are characterized by extremely slow soil formation from the underlying limestone, very shallow and patchy soil with a low water retention capacity, and high porosity of the underlying limestone rock (Zhu, 1997; Liu, 2009). Because of the shallow soils and highly porous limestone, drought events usually happen in such habitats. It has been reported that available soil water is only sufficient for plant transpiration needs for 7–14 days following rainfalls heavy enough to exceed soil field capacity (Zhou and Pan, 2001; Li et al., 2008). Drought stress is a crucial factor in limiting the survival, growth and distribution of plants in the karst habitats (Liu, 2009).

Osmotic adjustment in terms of accumulating compatible solutes has been considered as an important physiological adaptation for plant to resist drought (Morgan, 1984), which facilitate extracting water from dry soils and maintaining cell turgor, gas exchange and growth in very dry environments (White et al., 2000; Chaves et al., 2003). Soluble sugars and proline are two kinds of the most important compatible solutes in plants (Chaves et al., 2003; Ben Ahmed et al., 2009; Hessini et al., 2009). Besides their roles in osmotic adjustment, they may protect membranes from damages and stabilize the structures and activities of proteins and enzymes (Iyer and Caplan, 1998; Samuel et al., 2000; Villadsen et al., 2005; Lee et al., 2008; Ben Ahmed et al., 2009; Hessini et al., 2009).

Drought stress usually leads to oxidative stress due to stomatal closure (Lei et al., 2006; Ozkur et al., 2009), which causes the over-reduction of photosynthetic electron chain (Bacelar et al., 2007; Ben Ahmed et al., 2009) and high formation of reactive oxygen species (ROS) in chloroplasts and mitochondria (Asada, 1999; Fu and Huang, 2001). ROS including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (HO^-) and singlet oxy-

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Table 1
Plant species and some general information in this study.

Species	Code	Family	Growth form	Description
Pyracantha fortuneana (Maximowicz) H. L Li	PF	Rosaceae	Evergreen shrub	Evergreen shrub up to 3 m, occurring on open hill slopes. Its branches are heavily covered in thorns
Rosa cymosa Trattinnick	RC	Rosaceae	Deciduous shrub	Deciduous climbing or scandent shrub up to 5 m, occurring on open hill slopes. Its branches are scattered with hooked prickles
Cinnamomum bodinieri H. Léveillé	CB	Lauraceae	Evergreen tree	Evergreen tree up to 16 m, occurring in sparse forests in southern China
Broussonetia papyrifera (Linnaeus) L'Héritier ex Ventenat	BP	Moraceae	Deciduous tree	Deciduous tree up to 10 m, occurring in most regions of China. Its leaves are densely covered with hairs
Platycarya longipes Wu	PL	Juglandaceae	Deciduous tree	Deciduous tree up to 15 m. A common dominant in mixed forests on mountain slopes. It is restricted to limestone substrates
Pteroceltis tatarinowii Maximowicz	PT	Ulmaceae	Deciduous tree	Deciduous tree up to 20 m, occurring in the mountainous regions on limestone. Its leaves are covered by hairs

gen ($^1O_2$) could disrupt normal metabolisms of plants through oxidative damages to lipids, proteins, nucleic acids, and photosynthetic pigments and enzymes (Smirnoff, 1993; Fu and Huang, 2001; Ozkur et al., 2009). In order to overcome oxidative stress, plants have developed enzymatic and non-enzymatic antioxidant defense mechanisms to scavenge ROS (Smirnoff, 1993). The most important antioxidant enzymes are superoxide dismutase (SOD, EC 1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase (POD, EC 1.11.1.7). SOD converts O_2^- into H_2O_2 and O_2, and CAT and POD scavenge H_2O_2 into H_2O (Reddy et al., 2004; Yang et al., 2008; Wang et al., 2009). Besides, non-enzymatic antioxidative carotenoids (Car) such as β-carotene and xanthophylls can also quench ROS (e.g. $^1O_2$) and stabilize photosynthetic complexes (Adams et al., 1999; Bassi and Caffarri, 2000; Munné-Bosch and Peñuelas, 2003).

Deforestation has been one of the most serious environmental problems in the karst habitats, due to agricultural expansion, fuelwood collection, and livestock overgrazing (Liu, 2009). Many re-vegetation programs are not successful due to a lack of knowledge of the ecophysiological responses of native plants to drought stress. For instance, many tree species such as *Ligustrum lucidum* and *Pteroceltis tatarinowii*, which commonly occur in the healthy karst forests, are usually planted in badly degraded karst habitats such as shrublands (Zhang and Liu, 2005; Dai et al., 2008). The establishment of these tree seedlings is strongly limited by severe drought stress. Previous studies have reported many anatomical and physiological responses of karst plants to drought stress (Zhang et al., 2007; Chen et al., 2009; Wu et al., 2009; Zhu et al., 2009a; Liu et al., 2010). However, comparative studies on karst plant species which dominate in different degraded habitats with different drought intensities, for the purpose of evaluating their suitability for re-vegetation programs, is scarce. Moreover, the relationships between drought tolerance and accumulations of osmotic solutes, and between photosynthetic performance and activities of antioxidant enzymes in karst plants have seldom been reported.

In order to provide more detailed knowledge for the selection of plant species and contribute to the success of re-vegetation programs, we compared osmotic adjustment and antioxidant enzymes of two shrubs with four trees under experimental drought conditions. The two shrubs dominate in shrublands, while the four trees occur in healthy forests. It was previously reported that the two shrubs had higher photosynthetic capacities than the four trees under severe drought stress (Liu et al., 2010). We hypothesized that the two shrubs had higher capacities of osmotic adjustment and antioxidant defense than the four trees, which permitted their higher drought tolerance and better photosynthetic performance under drought stress.

2. Materials and methods

2.1. Plant materials and treatments

Six native woody species in the karst habitats were studied (Table 1): two shrubs, *Pyracantha fortuneana* and *Rosa cymosa*, and four trees, *Broussonetia papyrifera*, *Cinnamomum bodinieri*, *Platycarya longipes* and *P. tatarinowii*. Of the six species, *P. fortuneana* and *C. bodinieri* are evergreen, and the other four are deciduous. The two shrubs usually dominate in shrublands, growing in clusters, while *P. longipes* dominates in mixed evergreen and deciduous broad-leaved forests, accompanied by *B. papyrifera*, *C. bodinieri* and *P. tatarinowii*.

Two-year-old seedlings were grown individually in plastic pots (25 cm in diameter and 20 cm tall) filled with 2700 g clay-calcareous soil collected from the karst hills in Guizhou Province. Prior to drought stress treatments, all plants were well-watered. On May 15, 2008, 100 healthy plants of similar size for each species were randomly assigned to each of the four drought treatments as follows:

(1) well-watered (D1): the soil water potential was controlled at −0.1 MPa;
(2) mild drought stress (D2): the soil water potential was −0.5 MPa;
(3) moderate drought stress (D3): the soil water potential was −1.0 MPa;
(4) severe drought stress (D4): the soil water potential was −1.5 MPa.

During the experiment, which lasted for 100 days, the soil water potentials and corresponding soil water contents used in the study were calculated from soil water retention curves. The pots were kept at the designated drought stress levels by weighting. The study was carried out in a greenhouse at the Guizhou University in Guiyang, China. During the experiment, the minimum and maximum temperatures inside the greenhouse were 16.2 °C and 33.5 °C, respectively. The maximum photosynthetic photon flux density (PPFD) on sunny days in the nearby karst hills ranged from 900 to 1300 μmol m^{-2} s^{-1}, and the maximum PPFD inside the greenhouse was about 1000 μmol m^{-2} s^{-1}.

2.2. Water potential

Midday leaf water potential was measured on the youngest fully expanded leaves with a WP4-T Dewpoint PotentiaMeter (Decagon Devices, Inc., USA) between 12:00 and 14:30 h, on five individual plants per species for each treatment after 20 and 40 days of treatments.

2.3. Chlorophyll fluorescence

The maximum quantum efficiency of PSII photochemistry (F_v/F_m) was determined on the youngest fully expanded leaves during the morning (08:00–11:30 h), using an open gas exchange system (LI-6400; LI-COR, Inc., Lincoln, NE, USA) with an integrated fluorescence chamber (LI-6400-40 leaf chamber fluorometer; LI-COR). Following dark adaptation for 2 h, the minimum fluorescence (F_o) was determined by a measuring light of about 0.5 μmol photon m^{-2} s^{-1}, and the maximum fluorescence (F_m) was determined by a 0.8-s saturating flash of about 10,000 μmol photon m^{-2} s^{-1} in the dark-adapted leaves. F_v/F_m was calculated as $(F_m - F_o)/F_m$ (Maxwell and Johnson, 2000). Five replicates per species and treatment were randomly obstained from different individuals after 20 and 40 days of treatments.

2.4. Pigments

Total chlorophylls (Chl_{a+b}), chlorophyll *a* (Chl_a), chlorophyll *b* (Chl_b), and carotenoids (Car) were determined spectrophotometrically using 80% acetone as a solvent (Lichtenthaler, 1987). Three replicates per species and treatment were obtained from the youngest fully expanded leaves of different individuals during midday after 20 and 40 days of treatments.

2.5. Soluble sugars and proline

Soluble sugars were determined by the anthrone method (Yemm and Willis, 1954). Proline was determined by the ninhydrin method (Bates et al., 1973). Three replicates per species and treatment were obtained from the youngest fully expanded leaves of different individuals during midday after 20 and 40 days of treatments.

2.6. Cellular damages

Lipid peroxidation was measured in terms of malondialdehyde (MDA) (Hodges et al., 1999). To determine cell membrane stability and integrity, ion leakage was measured according to a simplified method (Nayyar, 2003). Leaves were thoroughly washed, cut into small discs and placed in vials filled with 10 ml deionized water. After incubation at 25 °C for 12 h in dark condition, the electrical conductivity (initial EC) in the bathing solution was determined by a conductivity meter (DDS-307, Shanghai Precision and Scientific Instrument LTD., Shanghai, China). Then the samples were heated at 100 °C for 20 min and the conductivity (final EC) in the bathing solution was read again. Three replicates per species and treatment were obtained from the youngest fully expanded leaves of different individuals during midday after 20 and 40 days of treatments.

$$\text{Ion leakage was defined as EC } (\%) = \left(\frac{\text{initial EC}}{\text{final EC}}\right) \times 100$$

2.7. Antioxidant enzymes assay

Superoxide dismutase (SOD) activity was determined by the nitroblue tetrazolium (NBT) method (Fu and Huang, 2001). One unit of SOD activity was defined as the amount of enzyme required to produce a 50% inhibition of reduction of NBT at 560 nm. Activities of catalase (CAT) and peroxidase (POD) were determined using the methods of Fu and Huang (2001). For CAT, the decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm for 1 min. For POD, the oxidation of guaiacol was measured by the increase in absorbance at 470 nm for 1 min. One unit of CAT and POD activity was defined as an absorbance change of 0.01 units per min. Total content of foliar protein was measured according to Bradford (1976), using bovine serum albumin as a standard. The activity of each enzyme was expressed on protein basis. Three replicates per species and treatment were obtained from the youngest fully expanded leaves of different individuals during midday after 20 and 40 days of treatments.

2.8. Leaf area ratio

At the end of experiment, five intact plants per species and treatment were randomly harvested for biomass and leaf area determinations. Total leaves of each plant were scanned into digital pictures with a scanner (Canon LiDE 25, Cannon Inc., Japan) and then leaf area was calculated from the pictures using Photoshop CS7.1 software (Adobe Systems Incorporated, USA). Each plant was dried to a constant weight in an oven at 85 °C to get the dry weight. Leaf area ratio (LAR; leaf area per total plant biomass) was also calculated.

2.9. Statistical analysis

All data were subjected to two-way analysis of variance (ANOVA) to determine differences among species and treatments for each variable at each sampling time. For each species, the values of each physiological variable were compared by repeated-measures analysis of variance (ANOVAR), with "drought intensity" as between-subject effects and "drought time" as within-subject effects. The significant differences between means were determined using Tukey's test at $P < 0.05$ level. Data were tested for normality by Kolmogorov–Smirnov test. When necessary, data were transformed to meet the assumptions of ANOVA. Linear regression coefficients between contents of osmotic solutes and activities of antioxidant enzymes were calculated. Statistical tests were performed with SPSS 13.0 (SPSS, Chicago, USA).

3. Results

3.1. Water potential

Under well-watered condition, midday water potentials were relative higher in *R. cymosa*, *C. bodinieri* and *B. papyrifera* than in the other three species (Fig. 1; $P < 0.05$). After 20 days of treatments, midday water potentials in all six species significantly decreased as drought stress intensified, with sharper decreases in *P. longipes* and *P. tatarinowii* than in the other four species under severe drought stress (Fig. 1; $P < 0.01$). Except for *P. fortuneana*, the prolonged drought treatments usually decreased midday water potentials in all six species, especially in *C. bodinieri* under severe drought stress (Fig. 1; $P < 0.01$). After 40 days of drought treatments, *P. fortuneana* and *R. cymosa* exhibited less reductions of midday water potential than the four trees (Fig. 1; $P < 0.01$).

3.2. Chlorophyll fluorescence

After 20 days of treatments, the values of F_v/F_m in all six species significantly decreased under severe drought stress, with higher values in *P. fortuneana* and *R. cymosa* than in the four trees (Fig. 2; $P < 0.01$). Prolonged severe drought treatments also caused a decline in F_v/F_m in *P. fortuneana*, *R. cymosa* and *B. papyrifera* (Fig. 2a, b, and d).

3.3. Pigments

Under well-watered condition, the values of Chl_{a+b} and Car were significantly higher in *B. papyrifera* and *P. longipes* than in the other four species (Figs. 3 and 4; $P < 0.01$). However, drought stress caused less decreases of Chl_{a+b} and Car in *P. fortuneana* and *C. bodinieri* than

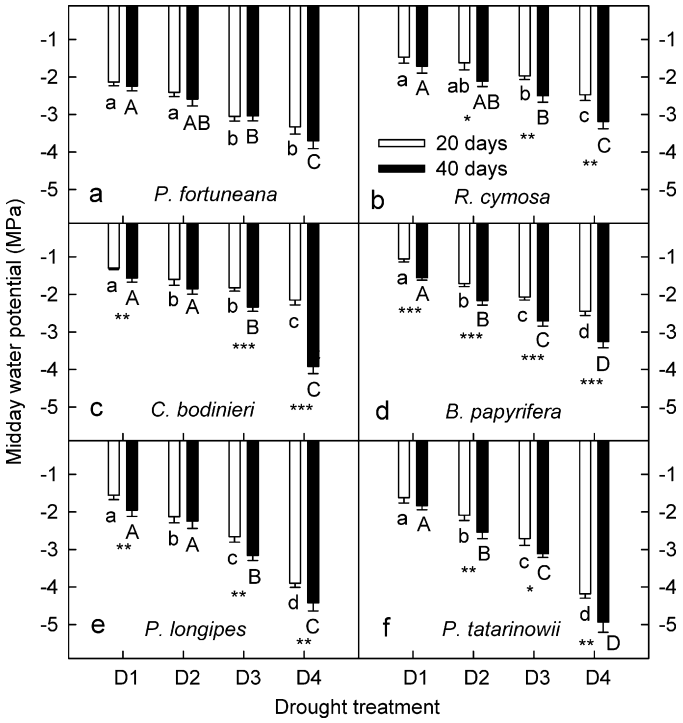

Fig. 1. Midday water potential of six karst species after 20 (open bars) and 40 (closed bars) days of four drought treatments (Mean ± SE; $n = 5$). *Drought treatments*: D1, well-watered; D2, mild drought stress; D3, moderate drought stress; D4, severe drought stress. *Shrubs*: *P. fortuneana* and *R. cymosa*, *Trees*: *C. bodinieri*, *B. papyrifera*, *P. longipes* and *P. tatarinowii*. Different letters denote significant differences among four drought treatments after 20 (lowercase letters) and 40 (uppercase letters) days of treatments ($P < 0.05$). Significant differences between two treatment times under each drought treatment: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

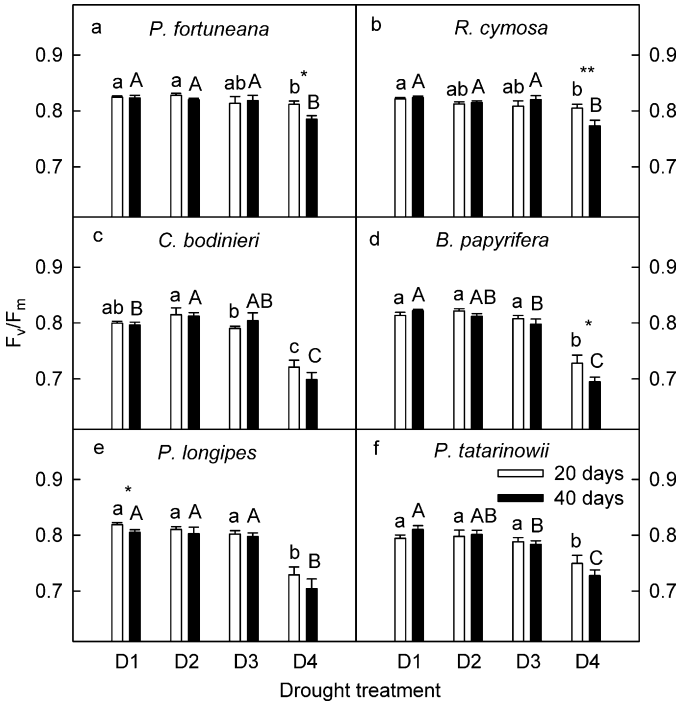

Fig. 2. The maximum quantum efficiency of PSII photochemistry (F_v/F_m) of six karst species after 20 and 40 days of four drought treatments (Mean ± SE; $n = 5$). Figure annotations are the same as in Fig. 1.

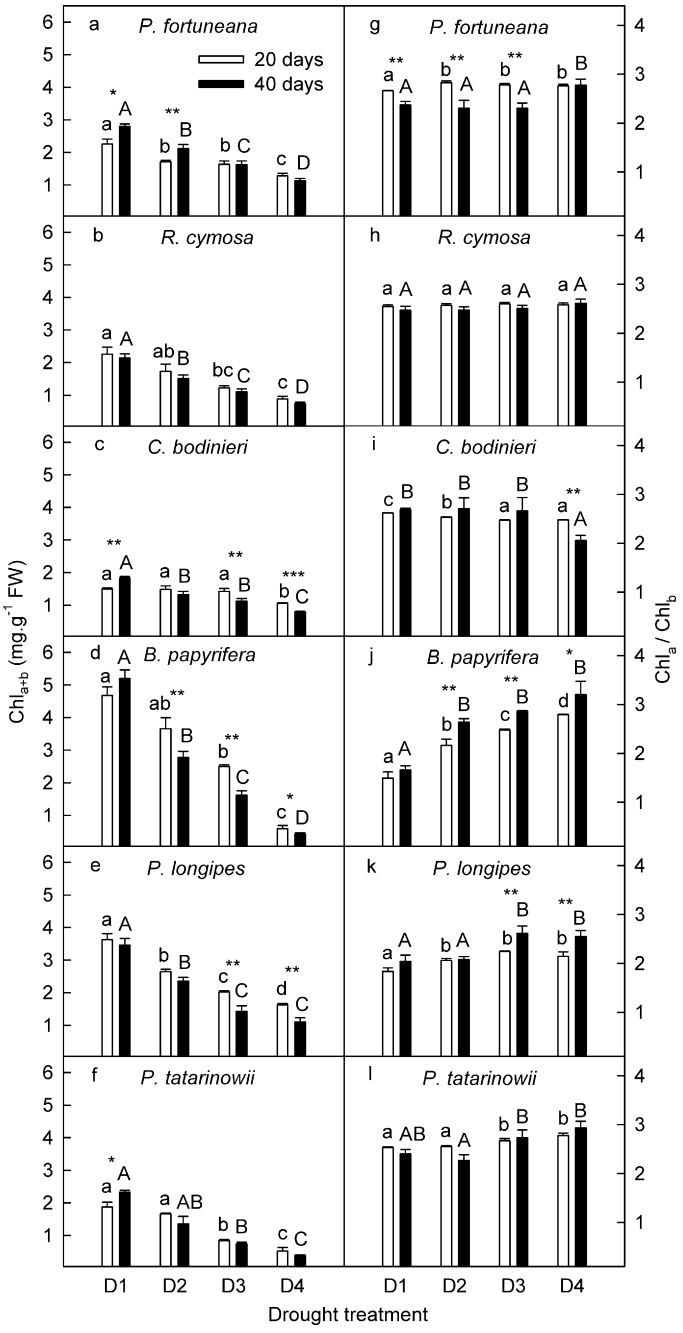

Fig. 3. Total chlorophylls (Chl_{a+b}) and the ratio of chlorophyll *a* to chlorophyll *b* (Chl_a/Chl_b) of six karst species after 20 and 40 days of four drought treatments (Mean ± SE; $n = 3$). Figure annotations are the same as in Fig. 1.

in the other four species after 20 days of treatments (Figs. 3 and 4; $P < 0.01$). Prolonged drought stress significantly decreased Chl_{a+b} in *C. bodinieri*, *B. papyrifera* and *P. longipes* (Fig. 3c–e) and also decreased Car in *P. longipes* and *P. tatarinowii* (Fig. 4e and f). As drought stress intensified, the ratio of Chl_a/Chl_b increased in *P. fortuneana*, *B. papyrifera*, *P. longipes* and *P. tatarinowii*, decreased in *C. bodinieri*, and did not change in *R. cymosa* (Fig. 3g–l). Prolonged drought treatments significantly increased Chl_a/Chl_b in *B. papyrifera* and *P. longipes* (Fig. 3j and k), but decreased that in *C. bodinieri* under severe drought stress (Fig. 3i). After 20 days of treatments, the ratio of Car/Chl_{a+b} in all six species significantly increased as drought stress intensified, except *C. bodinieri* and *P. tatarinowii* in which the value of Car/Chl_{a+b} increased after a longer time of drought treatments (Fig. 4g–l). Prolonged drought treat-

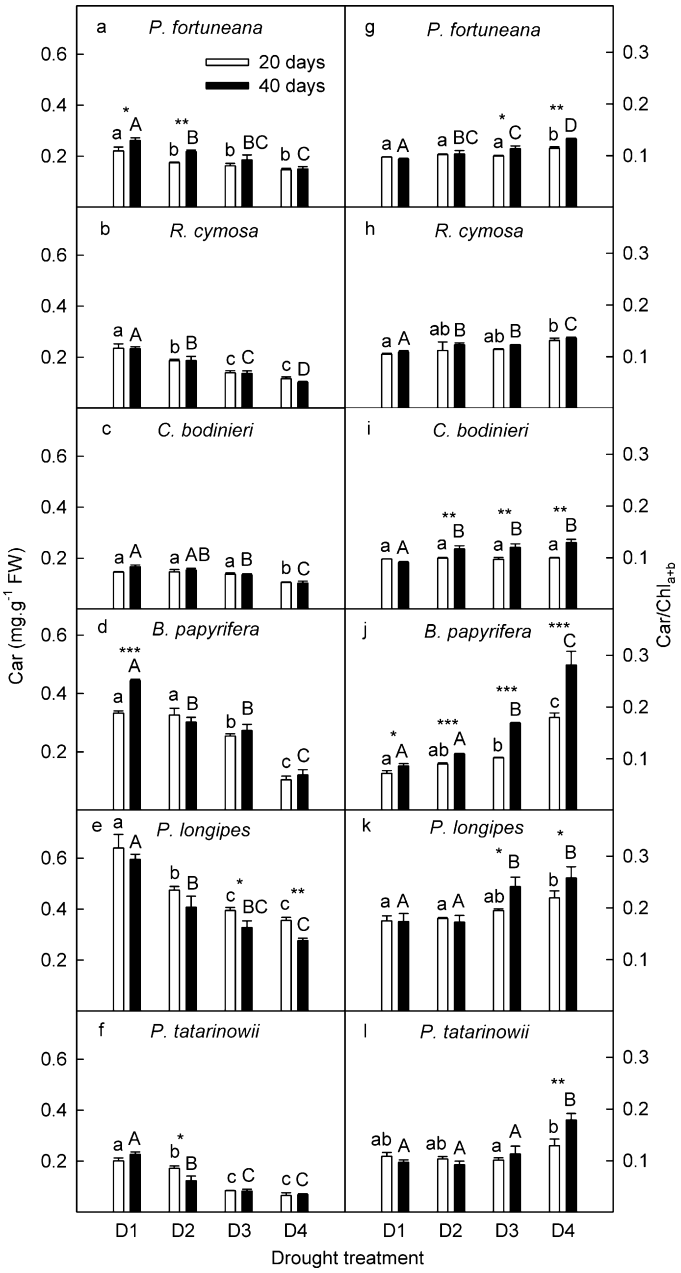

Fig. 4. Carotenoids (Car) and the ratio of carotenoids to total chlorophylls (Car/Chl_{a+b}) of six karst species after 20 and 40 days of four drought treatments (Mean ± SE; $n = 3$). Figure annotations are the same as in Fig. 1.

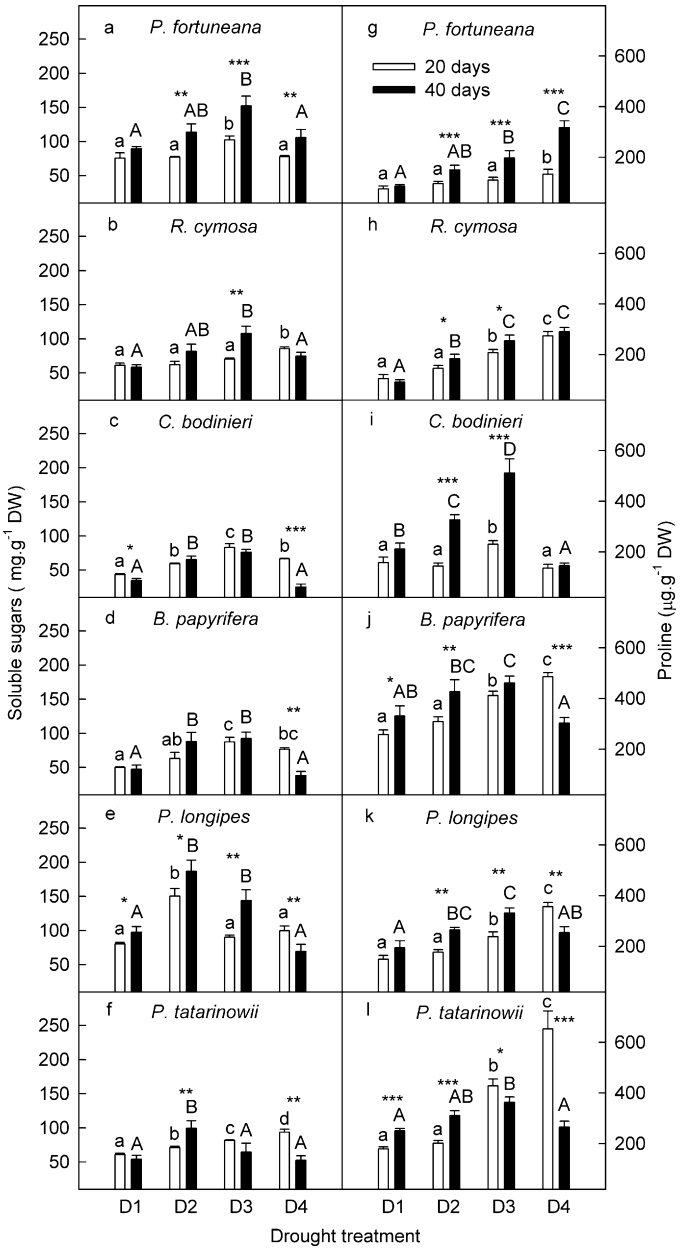

Fig. 5. The contents of soluble sugars and proline of six karst species after 20 and 40 days of four drought treatments (Mean ± SE; $n = 3$). Figure annotations are the same as in Fig. 1.

ments also increased the ratio of Car/Chl_{a+b} in *P. fortuneana*, *B. papyrifera* and *P. longipes* (Fig. 4g, j and k).

3.4. Soluble sugars and proline

Among the six species, the accumulation of soluble sugars exhibited different responses to drought intensities (Fig. 5a–f), exhibiting highest values in *P. fortuneana*, *C. bodinieri* and *B. papyrifera* under moderate drought stress, in *R. cymosa* and *P. tatarinowii* under severe stress, and in *P. longipes* under mild stress after 20 days of treatments. Usually, another 20 days of treatments tended to increase the accumulation of soluble sugars in *P. fortuneana*, *R. cymosa*, *P. longipes* and *P. tatarinowii* under mild and/or moderate stress, but decreased that in all six species under severe stress except *P. fortuneana* which showed an increase of soluble sugars content under prolonged severe drought stress (Fig. 5a–f). After 20 days of treatments, proline content in all six species gradually increased as drought stress intensified, except *C. bodinieri* in which proline content increased only under moderate stress (Fig. 5g–l). Prolonged drought treatments significantly increased proline content in all six species under mild and/or moderate stress, increased that in *P. fortuneana* under severe stress, but decreased that in *B. papyrifera*, *P. longipes* and *P. tatarinowii* under severe stress (Fig. 5g–l).

3.5. Cellular damages

After 20 days of treatments, MDA content and ion leakage in all six species gradually increased as drought stress intensified (Fig. 6). Prolonged drought treatments usually increased MDA content and ion leakage in all six species, except MDA content in *P. fortuneana* (Fig. 6). After 40 days of severe drought, *P. fortuneana*, *R. cymosa*

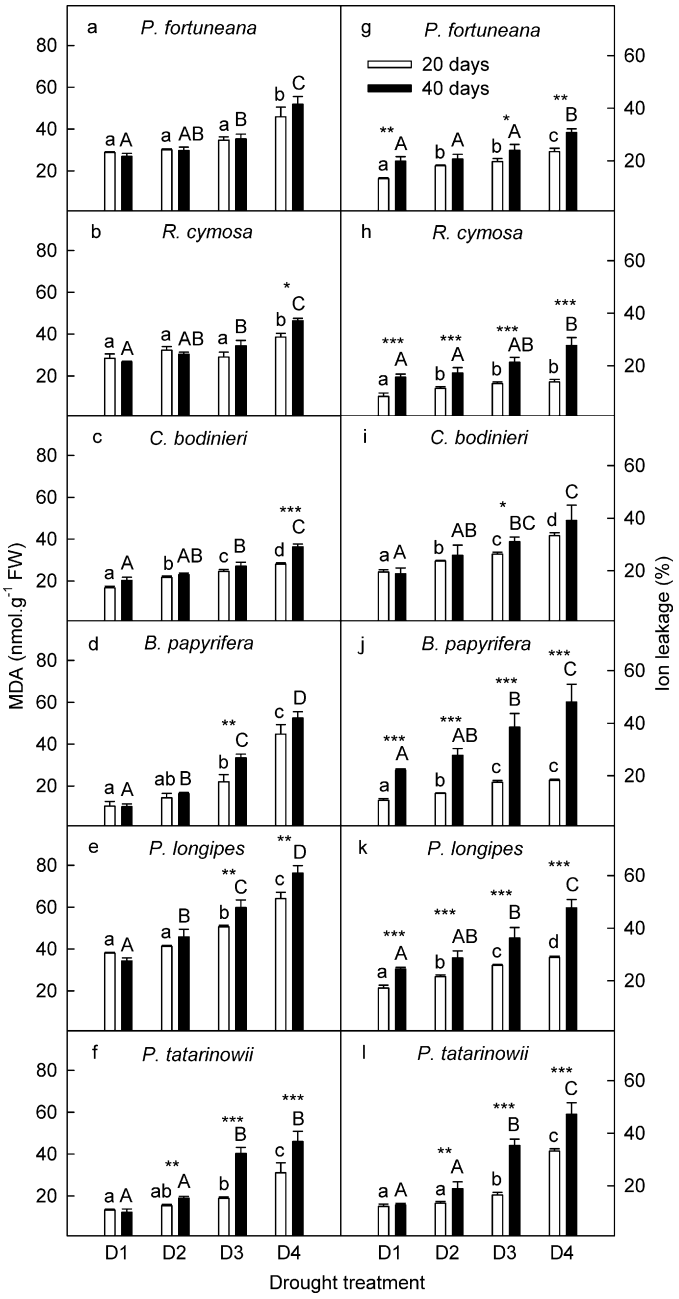

Fig. 6. Malondialdehyde (MDA) content and ion leakage of six karst species after 20 and 40 days of four drought treatments (Mean ± SE; $n = 3$). Figure annotations are the same as in Fig. 1.

and *C. bodinieri* exhibited lower increases of MDA content than the other three species (Fig. 6a–f; $P < 0.01$), and *P. fortuneana* and *R. cymosa* showed lower increases of ion leakage than the four trees (Fig. 6g–l; $P < 0.01$).

3.6. Antioxidant enzymes

After 20 days of treatments, drought stress increased SOD activity in all six species except *R. cymosa* and *C. bodinieri* (Fig. 7a–f). Prolonged drought treatments increased SOD activity in all six species except *P. longipes* under mild and moderate stress, increased that in *P. fortuneana* and *R. cymosa* under severe stress, but decreased that in *B. papyrifera*, *P. longipes* and *P. tatarinowii* under severe stress (Fig. 7a–f). After 20 days of treatments, CAT activity in *P. fortuneana*, *R. cymosa* and *P. longipes* gradually increased as drought intensified (Fig. 7g, h and k). However, CAT activity in *C. bodinieri* and *B. papyrifera* increased only under moderate stress (Fig. 7i and j) and in *P. tatarinowii* under mild stress (Fig. 7l). Prolonged treatments significantly increased CAT activity in all six species except *P. tatarinowii* under mild and/or moderate stress, but decreased that in *C. bodinieri* and *P. tatarinowii* under severe stress (Fig. 7g–l). After 20 days of treatments, POD activity showed highest values in *P. fortuneana*, *R. cymosa* and *B. papyrifera* under severe stress and in the other three species under moderate stress (Fig. 7m–r). Prolonged treatments increased POD activity in *P. fortuneana*, *C. bodinieri*, *B. papyrifera* and *P. tatarinowii* under mild and/or moderate stress, increased that in *P. fortuneana* under severe stress, but decreased that in *R. cymosa*, *C. bodinieri*, *B. papyrifera* and *P. tatarinowii* under severe stress (Fig. 7m–r).

3.7. Relationships between osmotic solutes and antioxidant enzymes

Proline content was linearly and positively correlated with SOD activity in all six species except *P. longipes* (Fig. 8a), was positively correlated with CAT activity in *R. cymosa*, *C. bodinieri* and *P. longipes* (Fig. 8b), and was positively correlated with POD activity in *P. fortuneana*, *R. cymosa*, *C. bodinieri* and *B. papyrifera* (Fig. 8c). Soluble sugars content was positively correlated with SOD activity in *P. fortuneana* and *B. papyrifera* (Fig. 8d) and was positively correlated with CAT and POD activities in *P. fortuneana*, *R. cymosa*, *C. bodinieri* and *B. papyrifera* (Fig. 8e and f). SOD activity was positively correlated with CAT activity in *P. fortuneana*, *R. cymosa*, *B. papyrifera* and *P. longipes* (Fig. 8g) and was positively correlated with POD activity in *P. fortuneana*, *C. bodinieri*, *B. papyrifera* and *P. longipes* (Fig. 8h). CAT activity was positively correlated with POD activity in *P. fortuneana*, *R. cymosa* and *C. bodinieri* (Fig. 8i).

3.8. Leaf area ratio

After 100 days of the experiment, the values of LAR were significantly lower in *P. fortuneana* and *R. cymosa* than in the four trees under well-watered condition (Table 2). LAR in all six species significantly decreased as drought stress intensified, with sharper decreases in the four trees than in the two shrubs. The two shrubs also exhibited lower LAR than the most of trees under severe drought stress (Table 2).

4. Discussion

After 40 days of drought treatments, the two shrubs (*P. fortuneana* and *R. cymosa*) exhibited higher values of F_v/F_m than the four trees (*C. bodinieri*, *B. papyrifera*, *P. longipes* and *P. tatarinowii*; Fig. 2) under severe drought stress. The higher photosynthetic efficiency of the shrubs than the trees may benefit from their better water status, as judged from their less reductions of midday water potential (Fig. 1).

Under drought conditions, the accumulations of proline and soluble sugars seemed to be associated with drought tolerance in many plant species. The rate of proline accumulation was significantly higher in drought-tolerant cultivars than drought-sensitive cultivars of wheat (Nayyar and Walia, 2003), mulberry (Reddy et al., 2004), and olive (Ben Ahmed et al., 2009). Soluble sugars also contributed to improving drought tolerance of peas (Sánchez et al., 1998), sugar beets (Choluj et al., 2008) and black poplars (Regier et al., 2009). In two mango cultivars, a cultivar, which exhibited more active accumulations of soluble sugars and proline, also revealed higher resistance to drought than the other one (Elsheery and Cao, 2008). In our study, proline content appeared to increase sharply in *B. papyrifera*, *P. longipes* and *P. tatarinowii* after 20 days of drought treatments (Fig. 5j–l), and in *C. bodinieri*

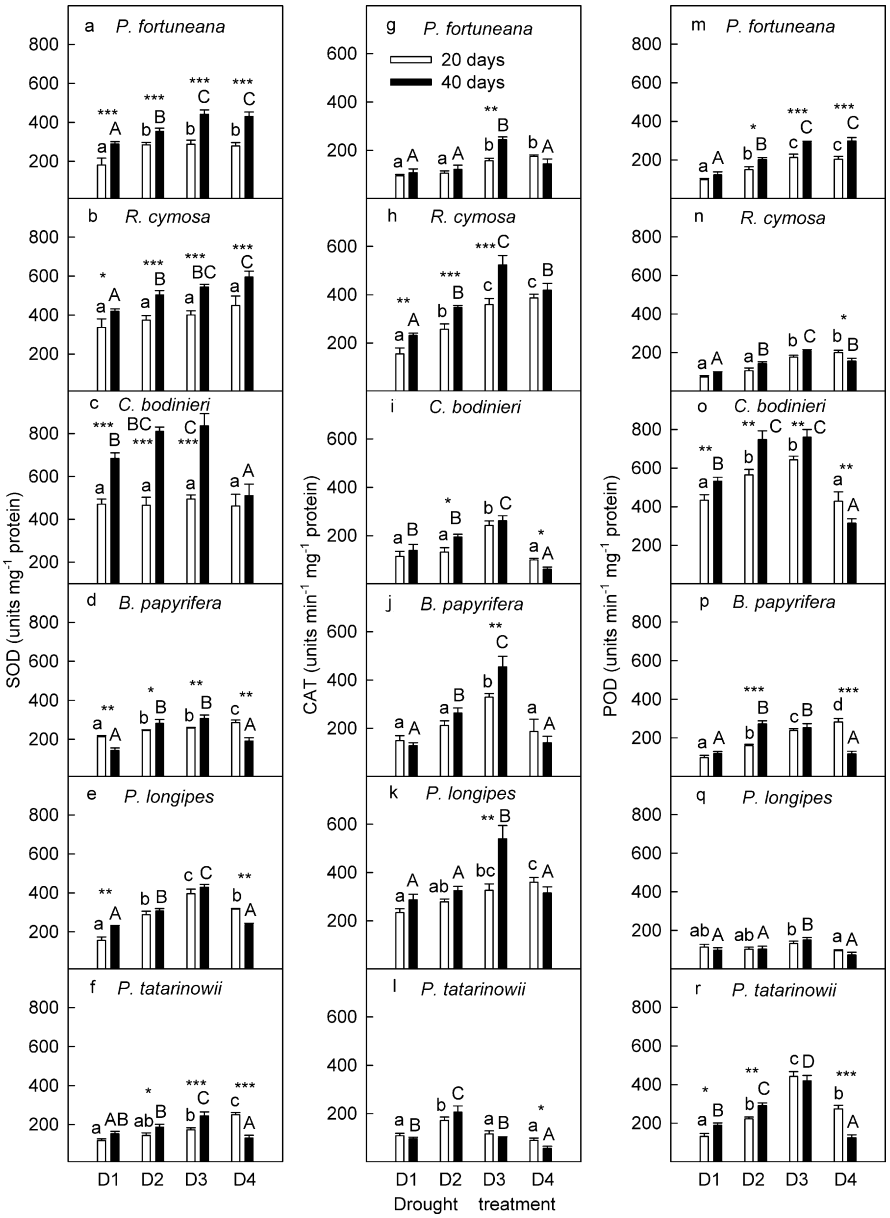

Fig. 7. Activities of superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) of six karst species after 20 and 40 days of four drought treatments (Mean ± SE; $n = 3$). Figure annotations are the same as in Fig. 1.

after prolonged mild and moderate drought stress (Fig. 5i). However, prolonged severe drought stress caused serous metabolic damages and largely decreased proline accumulation in the four tree species. On the contrary, although the two shrubs accumulated proline more slowly than the four trees as drought stress intensified, prolonged severe drought stress did not reduce proline content in *R. cymosa* (Fig. 5h) and even significantly increased that in *P. fortuneana* (Fig. 5g). The responses of soluble sugars to drought intensity showed the similar trends of proline between the shrubs and trees (Fig. 5a–f). There were significantly negative and linear relationships of midday water potential with proline ($y = -62.88x + 14.85$; $R^2 = 0.26$; $P = 0.043$) and soluble sugars contents ($y = -22.21x + 32.37$; $R^2 = 0.35$; $P = 0.016$) in the shrubs when pooling the data of *P. fortuneana* and *R. cymosa* together. These

Table 2
Leaf area ratio ($m^2\ kg^{-1}$) of six karst species after 100 days of four drought treatments (Mean ± SE; $n = 5$).

Species	Well-watered (D1)	Mild drought stress (D2)	Moderate drought stress (D3)	Severe drought stress (D4)
P. fortuneana	3.42 ± 0.23 a, C	2.72 ± 0.29 ab, CD	2.09 ± 0.22 bc, B	1.48 ± 0.18 c, B
R. cymosa	2.35 ± 0.37 a, C	2.00 ± 0.44 ab, D	1.60 ± 0.42 ab, B	1.23 ± 0.25 b, B
C. bodinieri	6.41 ± 0.28 a, A	5.31 ± 0.75 ab, A	3.60 ± 0.28 b, A	1.83 ± 0.61 c, AB
B. papyrifera	6.59 ± 0.87 a, A	3.87 ± 0.69 b, AB	2.58 ± 0.60 bc, AB	2.42 ± 0.32 c, A
P. longipes	5.32 ± 0.52 a, AB	3.61 ± 0.26 b, B	2.89 ± 0.36 bc, A	2.70 ± 0.16 c, A
P. tatarinowii	4.32 ± 0.37 a, B	3.40 ± 0.17 b, BC	2.08 ± 0.16 c, B	1.46 ± 0.51 c, B

Different lowercase letters in the same row denote significant differences among four drought treatments, while different uppercase letters in the same column denote significant differences among six species at $P < 0.05$.

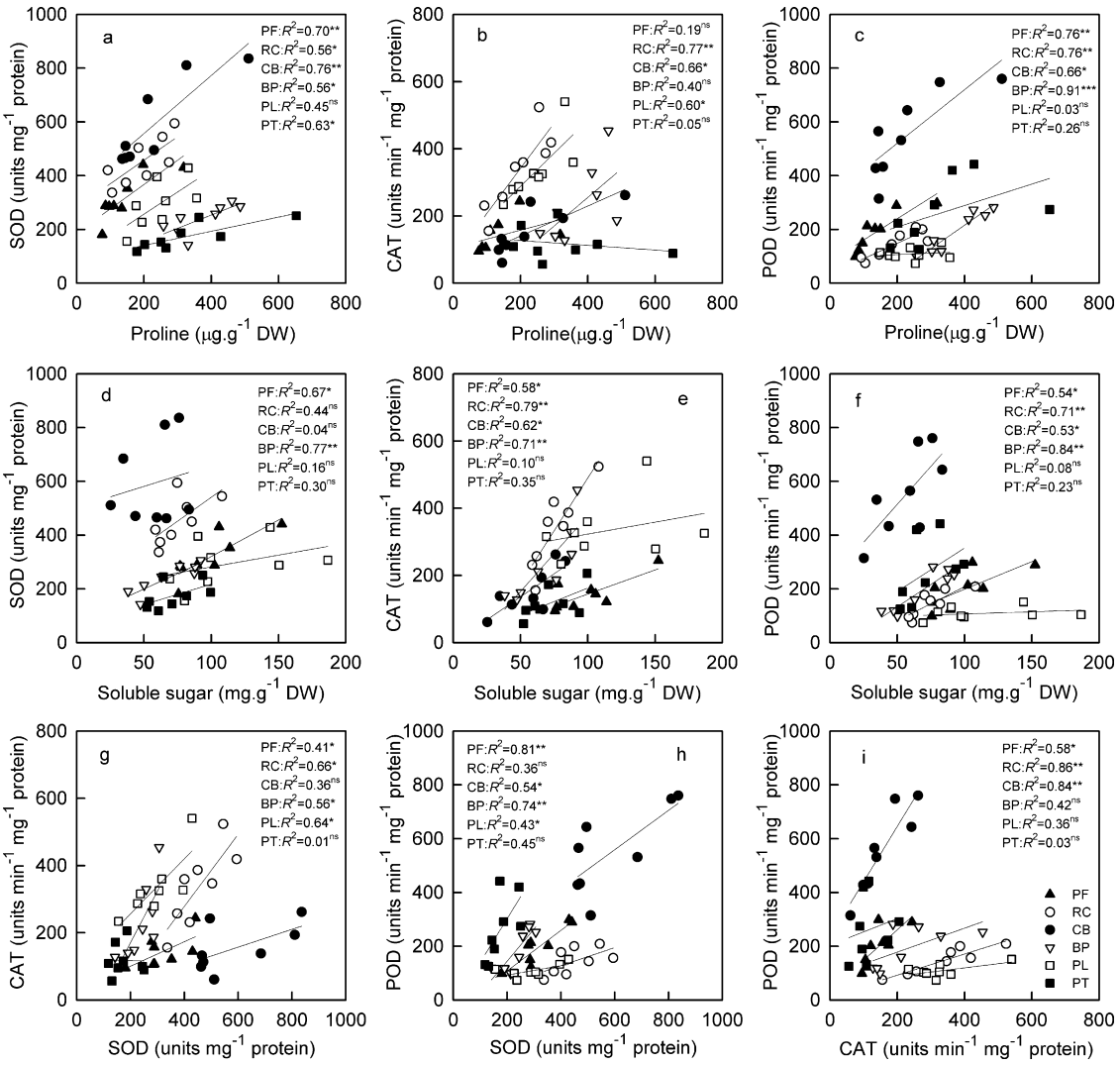

Fig. 8. Correlations between proline content and superoxide dismutase (SOD) activity (a), catalase (CAT) activity (b), peroxidase (POD) activity (c); between soluble sugars content and SOD activity (d), CAT activity (e), POD activity (f); between SOD activity and CAT activity (g), POD activity (h); and between CAT activity and POD activity (i). *Shrubs*: *P. fortuneana* (PF, ▲) and *R. cymosa* (RC, ○), *Trees*: *C. bodinieri* (CB, ●), *B. papyrifera* (BP, ▽), *P. longipes* (PL, □) and *P. tatarinowii* (PT, ■). Values are means of three replicates per species and treatment at two sampling times (error bars are omitted for clarity). The solid lines represent the best-fit linear regressions for each species: $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$; ^{ns}not significant.

results suggested that the two shrubs had higher capacity of osmotic adjustment in terms of accumulating proline and soluble sugars, especially under prolonged severe drought, which could maintain water absorption under such harsh conditions (White et al., 2000; Chaves et al., 2003). Besides the capacity of osmotic adjustment, the better water status of the two shrubs could be associated with their lower leaf area ratio than the trees (Table 2). Low leaf area ratio can reduce transpiration area, light harvesting and water loss, which would be especially important under drought stress conditions.

The ion leakage is an indicator of cell membrane stability and integrity, which is commonly considered as one of the best physiological components of drought tolerance in plants (Kocheva et al., 2004; Xu et al., 2008). MDA content is usually used to measure the extent of lipid peroxidation resulting from oxidative stress (Smirnoff, 1993). The increasing of MDA content was accompanied by an increase of ion leakage in all six species (Fig. 6), which indicated that lipid peroxidation led to membrane fluidity resulting in enhanced membrane permeability, as suggested by other authors (Lima et al., 2002; Reddy et al., 2004; Zhou et al., 2007). After a prolonged severe drought treatment, *P. fortuneana* and *R. cymosa* exhibited lower increases of ion leakage and MDA content than the four trees (Fig. 6), which indicated that the two shrubs had higher tolerance to severe drought stress.

Reduction of pigments content, as a result of either slow synthesis or fast breakdown, has been considered as a typical symptom of oxidative stress (Smirnoff, 1993). Under mild to moderate drought stress, the decreases of pigments content (Chl_{a+b} and Car; Figs. 3 and 4) in all six species did not cause any decrease of F_v/F_m (Fig. 2), which indicated that pigments breakdown was not accompanied by the decreasing of the maximum photochemical efficiency. Other authors explained this phenomenon as a photoprotection mechanism through reducing light absorbance by decreasing pigments content (Munné-Bosch and Alegre, 2000; Galmés et al., 2007; Elsheery and Cao, 2008). Higher ratio of Chl_a/Chl_b was also considered as a decreased emphasis on light collection in relation to the rates of PSII photochemistry (Demmig-Adams and Adams, 1996). As drought stress intensified, the most of studied species increased the ratio of Chl_a/Chl_b (Fig. 3g–l), which could be explained as a decrease of peripheral light-harvesting complexes. Since Car played an important role in photoprotection (Demmig-Adams and Adams, 1996; Adams et al., 1999; Munné-

Bosch and Peñuelas, 2003), the increased ratio of Car/Chl_{a+b} in all six species under drought conditions (Fig. 4) indicated a higher need of photoprotection by Car (Baquedano and Castillo, 2006; Elsheery and Cao, 2008).

The higher values of F_v/F_m and lower increases of MDA and ion leakage in the two shrubs than in the four trees after prolonged severe drought stress were attributed to higher activities of antioxidant enzymes in the shrubs. In the current study, severe drought stress and/or prolonged severe drought largely decreased the activities of antioxidant enzymes in *C. bodinieri*, *B. papyrifera*, *P. longipes* and *P. tatarinowii* (Fig. 7), indicating that the scavenging function of antioxidant enzymes was impaired by severe stress (Fu and Huang, 2001). On the contrary, in response to prolonged severe drought, CAT activity in the two shrubs did not decrease (Fig. 7g and h), and SOD activity in the two shrubs (Fig. 7a and b) and POD activity in *P. fortuneana* (Fig. 7m) even significantly increased. Under drought conditions, the activities of SOD, CAT and ascorbate peroxidase (APX, EC 1.11.1.11) increased to a greater extent, resulting in lower levels of lipid peroxidation and electrolyte leakage, in a drought-tolerant clone than in a drought-sensitive one of *Coffea Canephora* (Lima et al., 2002). The drought-resistant *Phaseolus acutifolius* also revealed higher activities of SOD, CAT, POD and APX, and lower level of lipid peroxidation than the drought-susceptible *P. vulgaris* (Türkan et al., 2005). Khanna-Chopra and Selote (2007) attributed lower membrane injury to the higher activities of POD and APX in a drought-tolerant wheat cultivar than in a drought-sensitive cultivar under severe drought stress. High activities of antioxidant enzymes also improved drought tolerance of cultivars of mulberry (Reddy et al., 2004), tea (Upadhyaya et al., 2008) and olive (Ben Ahmed et al., 2009). It seemed to be that higher activities of antioxidant enzymes provided higher protection against oxidative stress in the two shrubs than in the four trees under severe drought stress, as judged from lower increases of MDA and ion leakage in the shrubs.

SOD controls the first threshold of the water–water cycle of antioxidant system (Asada, 1999; Zhu et al., 2009a). It plays a key role in quenching active oxygen (Fu and Huang, 2001), working as catalyzing the dismutation of O_2^- into H_2O_2 which are eliminated by CAT, POD and other antioxidant enzymes. The observed positive correlations among activities of SOD, CAT and POD in the studied species (Fig. 8g–i) suggested that the increase of SOD activity was accompanied by increases of CAT and POD activities as a result of high demand of quenching H_2O_2. The intimate relationships between enhanced or constitutive antioxidant enzyme activities in response to drought stress were also observed in many other species (Türkan et al., 2005; Chen and Cao, 2008; Zhu et al., 2009b). The positive relationships between contents of osmotic solutes (proline and soluble sugars) and antioxidant enzyme activities (SOD, CAT and POD) were also observed in our study (Fig. 8a–f). It was reported that proline accumulation could activate the antioxidant defense mechanisms (Türkan et al., 2005; Ben Ahmed et al., 2009). Since proline and soluble sugars could stabilize the structures and activities of enzymes (Chaves et al., 2003), the high accumulations of proline and soluble sugars in the two shrubs under prolonged severe drought stress may largely permit their high activities of antioxidant enzymes.

Plant resistance to drought stress has been classically divided into avoidance and tolerance strategies (Chaves et al., 2003). Under well-watered condition, the three deciduous trees exhibited high pigments content and leaf area ratio for maximizing carbon assimilation. As drought stress intensified, the deciduous trees revealed worse water status and higher levels of cellular damages than the two shrubs under prolonged severe drought due to their lower capacities of osmotic adjustment and antioxidant protection. In response to drought stress, the three deciduous trees also exhibited much sharper decreases of pigments content and leaf area ratio than the two shrubs for minimizing light harvesting and water loss. It was reported that the three deciduous trees could rapidly recovered from severe drought stress and showed enhanced photosynthetic capacities after rewatering (Liu et al., 2010), which could be explained by that shedding leaves coupled with pigments breakdown was not only for avoiding drought periods but also for protecting the perennial parts of deciduous plants (Taulavuori et al., 2010). The three deciduous trees seemed to be more sensitive to water availability and used avoidance strategies. On the contrary, after prolonged severe drought stress, the two shrubs exhibited better water status, less extent of lipid peroxidation and higher photosynthetic efficiency than the four trees, which could be explained by the higher accumulations of solutes and higher activities of antioxidant enzymes in the two shrubs than in the four trees. This indicated that the two shrubs employed tolerance strategies against drought stress. The evergreen tree, *C. bodinieri*, mostly behaved as a tolerant species, e.g. showed small reductions of water potential, low cellular damages and small reductions of pigments content under prolonged moderate drought stress due to the high proline accumulation and high activities of SOD and POD. However, under prolonged severe stress, the large reductions of water potential, very low F_v/F_m and high levels of cellular damages indicated *C. bodinieri*'s weak tolerance to severe drought.

5. Conclusion

As drought stress intensified, the two shrubs (*P. fortuneana* and *R. cymosa*) exhibited higher F_v/F_m, less reductions of midday water potential, and lower increases of MDA content and ion leakage than the four trees (*C. bodinieri*, *B. papyrifera*, *P. longipes* and *P. tatarinowii*). Drought stress decreased pigments content but increased the ratio of Car/Chl_{a+b} in the studied species. Prolonged severe drought stress largely decreased accumulations of osmotic solutes and activities of antioxidant enzymes in the four trees, but significantly increased proline content and superoxide dismutase activity in the two shrubs and peroxidase activity in *P. fortuneana*. The positive relationships were observed among activities of antioxidant enzymes, and between contents of osmotic solutes and activities of antioxidant enzymes. These results suggested that the two shrubs had higher tolerance to severe drought stress than the four trees due to higher capacities of osmotic adjustment and antioxidant protection. The site-species matching work seemed to be very important and can contribute to the success of re-vegetation programs.

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