***Pak. J. Bot.*, 50(4): 1335-1342, 2018.**

LEAF PHYSIOLOGICAL AND ANATOMICAL CHARACTERISTICS OF TWO INDICATOR SPECIES IN THE LIMESTONE REGION OF SOUTHERN CHINA UNDER DROUGHT STRESS

YONGBIN WU[1#], WENJUN HONG[2#] AND YOUHUA CHEN[3*]

[1]South China Limestone Plants Research Center, College of Forestry and Landscape Architecture, South China Agricultural University, Guangzhou, China
[2]Sanya Academic of Forestry, Sanya, Hainan, 572000, China
[3]Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu, 610041, China
[*]Corresponding author's emails: correspondence: haydi@126.com
 # Authors equally contribute to this work

Abstract

Drought is a critical limiting factor of plant growth, and plants living in arid areas must develop adaptation mechanisms to resist drought stress. In this study, we studied the drought resistance mechanisms of two tree species, *Triadica rotundifolia* and *Cinnamomum burmannii*, based on their foliar physiological and anatomical characteristics. These trees are indicator plants in the limestone region of southern China. We evaluated and compared the changes in the contents of chlorophyll, soluble protein, soluble sugars, proline, malondialdehyde (MDA), proline, activity of superoxide dismutase (SOD) and peroxidase (POD), chlorophyll fluorescence parameters, and leaf anatomical structure between these two species. The results showed the following. (1) The content of Chl_{a+b}, soluble protein, soluble sugar and SOD activity of the two species' seedlings had an increasing trend at first and then a decrease over the entire drought experiment. The change in MDA content was more sensitive in *T. rotundifolia*, and the peak (33.05 mmol·ml^{-1}) appeared on the 28th day of the drought experiment. Comparatively, the proline content and POD activity were more sensitive in *C. burmannii*, and the proline peak (217.11 μg·g^{-1}) appeared on the 24th day. (2) Under the continuous drought stress, the electron transport rate of the seedlings decreased, whereas the non-photochemical quenching showed different trends for the different species. Severe drought stress in *T. rotundifolia* caused an increasing trend in the light energy capture efficiency (F_v/F_m), while it declined continuously in *C. burmannii*. (3) Under the drought stress, the leaves of *T. rotundifolia* became thicker. The arrangement of spongy tissues became loose, and the ratio of palisade to spongy tissue decreased. However, the leaves of *C. burmannii* showed a few different features, such as inconspicuous differentiation between palisade and spongy tissues, a high loose degree of leaves, and low packing. (4) The leaf physiological indices had a strong relationship with the anatomy characteristics, as revealed by principal component analysis (PCA); the adaptability to drought tolerance of *T. rotundifolia* was stronger than *C. burmannii*. In conclusion, *T. rotundifolia* is better able to adapt to drought conditions due to its physiological and anatomical characteristics, which allow the species to better buffer drought-induced physiological responses and variability in leaf structure.

Key words: Limestone region, Drought stress, Stress-resistance physiology, Leaf anatomy.

Introduction

Limestone ecosystems are vulnerable around the world (Ren, 2005). The key features of limestone ecosystems include extremely slow soil formation from underlying limestone, poor water retention, and high-frequency drought microclimates. These features are all limiting factors on the growth of plant seedlings (Liu *et al.*, 2010). In limestone regions, plants usually suffer from frequent drought events, even in rainy seasons. This is caused by the shallow and highly porous limestone soils in combination with high evaporation rates. Studies have shown that available soil water is only sufficient for plant transpiration needs for 7-14 days following heavy rainfalls to exceed the soil field capacity. Therefore, tree species might be vulnerable, and their survival may be threatened in drought environments of the limestone regions (Rong *et al.*, 2012). Nevertheless, many plant taxa in the limestone ecosystem are endemic (Zhu *et al.*, 2003; Clements *et al.*, 2006; Wu *et al.*, 2010). How can these endemic plants adapt to drought stress conditions? This remains an open question.

Recent studies have focused on the physiological, morphological, and structural features of plant species surviving in limestone regions to investigate their adaptive mechanisms in response to drought stresses. Drought-resistant vascular plants in the limestone regions usually rely on the following ecological mechanisms to adapt: (1) high water utilization efficiency (Fu & Huang, 2004); (2) high physiological and biochemical performance (e.g., antioxidant enzymes and osmotic adjustments) (Mo *et al.*, 2008; Yao *et al.*, 2010; Dong *et al.*, 2011); (3) high photosynthetic performance (e.g., maximum net photosynthetic efficiency and effective thermal dissipation) (Maxwell & Johnson, 2000; Dong *et al.*, 2011); and (4) a shift in the leaf anatomical structure (Fu & Huang, 2004).

Triadica rotundifolia is a deciduous tree species of *Triadica* (Euphorbiaceae) and is mainly distributed in the Yunnan, Guizhou, Guangxi and Guangdong provinces of China (Li *et al.,* 2007). Nevertheless, it has calcium, drought resistance, and a strong adaptation to limestone habitats (Mo *et al.,* 2008; Zhang *et al.,* 2009). *Cinnamomum burmannii*, by contrast, is an evergreen tree species of *Cinnamomum* (Lauraceae), and it mostly grows in calcareous soil and is common in the secondary forests of the limestone region (Wu, 2004). Most previous studies have focused on analyzing the community structure and physiological and morphological traits related to the photosynthesis for *T.*

rotundifolia (Zhang *et al.*, 2009; Xu *et al.*, 2013; Hong *et al.*, 2015), whereas the chemical composition, cutting propagation, community structure, and physiology were examined for *C. burmannii* (Li *et al.*, 2007; Huang *et al.*, 2011; Tan *et al.*, 2011). To be best of our knowledge, little is known about how the leaf structure maintains growth and resists drought conditions (Catoni *et al.*, 2012; Nardini *et al.*, 2012; Pinto-Marijuan & Munne-Bosch, 2013).

To date, it has not been clearly elucidated how the physiological and anatomical structure of tree species change to adapt for drought condition in the limestone region. Assessing the drought-tolerant characteristics would deepen the understanding on how the appropriate artificial reforestation activities can help the restoration of forest habitats in the limestone region in southern China. In this study, the physiological and photosynthetic performances associated with the leaf structure were studied in two species at different drought treatments. We addressed the following questions: (1) How did the physiological and photosynthetic characteristics change when the drought intensity vary? (2) What were the main factors limiting growth under different drought conditions? (3) How was the relationship between physiological indices and leaf anatomy occur under different drought intensities?

Plant material and growth conditions: Two-year-old seedlings of *T. rotundifolia* and *C. burmannii* were planted individually in plastic pots (26 cm in diameter and 21 cm tall) filled with 4,000 g containing 2:1:1 (v/v) clay, perlite and sand, with slow release N:P:K (14:13:13) fertilizer that contained micronutrients. The soil field capacity was 29.54%, and the bulk density was 1.38 g·cm^{-3}. Ninety healthy seedlings of similar size for each species (Table 1) were assigned for the drought treatments.

Table 1. Summary statistics on the seedlings of two species used in our experiment. Data were presented as the mean ± SE (sample size: n=30).

Species	Height/cm	Base diameter /mm	Crow width/ cm
T. rotundifolia	53.58±10.85	7.66±1.44	30.24±3.25
C. burmannii	62.45±3.27	5.96±1.12	35.36±2.78

The experiment was carried out in a greenhouse at the South China Agriculture University, Guangzhou, China (23°06′26″N, 113°18′26″E). This area has a mixed tropical and subtropical climate. The summer season is long, hot and wet, with three-quarters of the annual rainfall occurring from May to September. The winters are cool and dry, and the minimal temperature generally remains above 13.3°C. The annual temperature reaches 21.8°C, and the annual rainfall is 1 800 mm. During the experiment, the minimum and maximum temperatures inside the greenhouse were 24.5°C and 36.5°C, respectively.

Experimental design: On June 13, 2015, 60 seedlings of the two studied species (each species had 30 seedlings) in the field were supplied with ample water and moved to the greenhouse for the drought experiment. The soil moisture in the field without artificial watering on the same day was measured and used as the control (i.e., field capacity). All seedlings of both species were subject to natural drought without artificial watering for subsequent days in the greenhouse. On the 0th, 4th, 8th, 12th, 16th, 20th, 24th and 28th days since June 14th, 3 leaf samples from 3 seedlings of each species were collected for laboratory tests. The soil moisture in the plastic pots was measured at each time when leaf sampling was conducted and is reported in Table 2. The proportion of field capacity was calculated as a percentage rate of the soil moisture in the pots with respect to the soil field capacity (i.e., 29.54%).

At each time of leaf sampling, the chlorophyll content (chlorophyll a: Chl_a, chlorophyll b: Chl_b, sum of chlorophyll a and b: Chl_{a+b}; ratio between chlorophyll a and b: $Chl_{a/b}$), soluble protein, soluble sugar, proline, malondialdehyde (MDA), superoxide dismutase (SOD) and peroxidase (POD) of the seedlings were determined in the lab (Chen & Wang, 2002). In general, a normal drought level (CK) was indicated if the soil moisture in the pots, with seedlings planted in the greenhouse, was greater than 80% of the field capacity (i.e., 29.54%); 50~70% soil moisture indicated light drought (LD), 30~50% represented moderate drought (MD), and less than 30% represented severe drought (SD) (Han & Zhao, 2010).

Additionally, in the CK, LD, MD and SD treatments, selected samples of leaves were fixed in an FAA solution (5 ml formalin: 5 ml acetic acid: 90 ml anhydrous alcohol), and their anatomical structure was studied. In addition, the chlorophyll fluorescence parameters were measured according to each drought stress.

Table 2. Soil moisture of two species under different drought treatments.

Drought stress (day)	Soil moisture (%)		Proportion of field capacity (%)		Water gradient
	Triadica rotundifolia	*Cinnamomum burmannii*	*Triadica rotundifolia*	*Cinnamomum burmannii*	
0	32.67	33.24	110.6	112.53	Normal level (CK)
4	26.54	25.60	89.84	86.66	
8	19.85	18.56	67.2	62.83	Light drought stress (LD)
12	15.34	14.24	51.93	48.21	
16	12.49	11.17	42.28	37.81	Moderate drought stress (MD)
20	10.02	9.55	33.93	32.33	
24	8.25	7.37	27.93	24.95	Severe drought stress (SD)
28	5.16	4.49	17.47	15.20	

Chlorophyll fluorescence: The following chlorophyll florescence parameters were determined and calculated according to Maxwell & Johnson (2000). The maximum quantum efficiency (F_v/F_m) of photosystem II (PSII) was calculated as (F_m-F_0)/F_m; the electron transport rate *ETR* was calculated as ((F_m'-F_0')/F_m' × PPFD ×0.5 × 0.84; and the non-photochemical quenching *NPQ* was computed as (F_m- F_m')/ F_m'. Here, the values of maximal (F_m) and minimal fluorescence (F_0) were the maximum and steady-state fluorescence in the light-adapted leaves (with PPFD at 1 500 μmol m^{-2} s^{-1}), and F_m' and F_v' were the maximal and minimal fluorescence after far-red illumination in the light-adapted leaves when the actinic light was removed. PPFD represented the photosynthetic photon flux density. To calculate *ETR*, a leaf absorptance of 0.85 and an equal distribution of excitation between PSI and PSII were assumed (Maxwell & Johnson, 2000).

Anatomical features: Tissue dehydration of leaf samples was carried out in alcohol by the following procedure: 50% alcohol for 1/2 h, 70% alcohol for 1/2 h, 90% alcohol for 1 h, 100% for 1 h and 100% for 1 h. This was followed by infiltration and final embedment in Spurr's resin. Cross- and paradermal sections for light microscopy (10 μm thick) were obtained in a Reichert Om U2 ultramicrotome and stained with toluidine blue stain (1% toluidine blue O in 1% borax solution), and digital photomicrographs of the section were taken under a light microscope (Leica DM 2500, Germany).

The resultant digital photos were scaled and analyzed with the ImageJ software (freely available from https://imagej.nih.gov/ij/). We measured the thickness (μm) of the cross-sectional leaf, distance between thickness on a leaf, upper and lower epidermal thicknesses, palisade parenchyma, and spongy parenchyma on a view of each section at magnifications of 200 × and 400 ×. From these measurements, we calculated the following parameters: tightness of leaf tissue (=the length of palisade parenchyma / leaf thickness × 100%) and porosity of leaf parenchyma (=the length of spongy parenchyma / leaf thickness × 100%).

Statistical analyses: All data are summarized as the means ± standard errors (SE) of three replications. The treatment effects were carried out with a one-way analysis of variance (ANOVA), and Tukey's Honestly Significant Difference (HSD) test at the 5% level was used to determine the statistical significance between treatments. The physiological indices, chlorophyll fluorescence and anatomical features were analyzed and summarized using principal component analysis (PCA). All data (except PCA) were analyzed by Statistical Package for the Social Science (SPSS) for Windows Release 19.0, and PCA was run with the Canoco 4.5 software (Centre for Biometry, Wageningen, Netherlands).

Results

Chlorophyll content: During the drought stress periods, the values of Chl_b and Chl_{a+b} in *T. rotundifolia* were not affected significantly by drought treatments at the beginning, but they decreased significantly as the drought intensity increased (Fig. 1B, C). In contrast, there was no obvious difference in the values of Chl_a throughout the drought experiment (Fig. 1A), whereas $Chl_{a/b}$ increased significantly and monotonically over time until the end of the drought experiment (i.e., on the 28th day) (Fig. 1D).

The Chl_a, Chl_b, and Chl_{a+b} in *C. burmannii* showed a trend of a significant increase at first, followed by a decrease under continuous drought stress. The peak was reached on the 12th day during the drought treatment (Fig. 1A-C). In contrast, the values of $Chl_{a/b}$ were not affected significantly as the experiment proceeded (Fig. 1D).

SOD and POD activities: The SOD activities of the two species showed a similar increasing trend at first, followed by a decrease under continuous drought stress. The highest SOD activity in both species was observed on the 12th day of the drought experiment (Table 3). However, the POD activity of *T. rotundifolia* had lower values and was in the range of 19.72 U g^{-1} min^{-1} to 84.15 U g^{-1} min^{-1}, while *C. burmannii* was in the higher values in the range of 406.11 U g^{-1} min^{-1} to 1 488.15 U g^{-1} min^{-1}. Under the drought period, the POD activities of the two species also maintained high values during the beginning period, but they seemed to decrease following the drought intensities previously subjected to the drought treatments (Table 3).

Osmoticum content and MDA content: The contents of soluble protein content and soluble sugar in the two species were affected significantly by drought treatments at the beginning and then decreased in the subsequent drought stress. The highest soluble protein content of *T. rotundifolia* was observed on the 12th day of drought treatment, and the highest value in *C. burmannii* was found on the 4th day of the experiment (Fig. 2A). The change in soluble sugar content was more sensitive in *T. rotundifolia*, and the peak (54.42%) appeared on the 12th day after treatment. The content of soluble sugar ranged from 18.84% to 56.51% in *C. burmannii* and was higher after the 16th drought day (Fig. 2B).

During the drought period, the proline content in the two species increased significantlyasthe drought stress increased. Under the severe drought condition, the proline content of *C. burmannii* was higher than that of *T. rotundifolia* (Fig. 2C).

Different MDA content patterns were found between the studied species under different drought stress periods. The MDA content in *T. rotundifolia* increased progressively as the drought stress was intensified; the peak (33.05 mmol·ml^{-1}) appeared on the 28th day after treatment (Fig. 2D). Nevertheless, the MDA content of *C. burmannii* increased significantly at the beginning and decreased significantly during the severe drought. During the drought period, the change in MDA content over time was higher in *T. rotundifolia* than it was in *C. burmannii*, except under the normal level treatment.

Chlorophyll fluorescence: The value of F_v/F_m in *T. rotundifolia* did not significantly change during the periods of drought treatments and generally maintained a high value in the mild drought treatment. However, the drought did intensify the decrease in F_v/F_m of *C. burmannii*, especially after drought treatment. The change in F_v/F_m in *Triadica rotundifolia* was not more sensitive than *C. burmannii*, showing a strong ability to capture light (Fig. 3A).

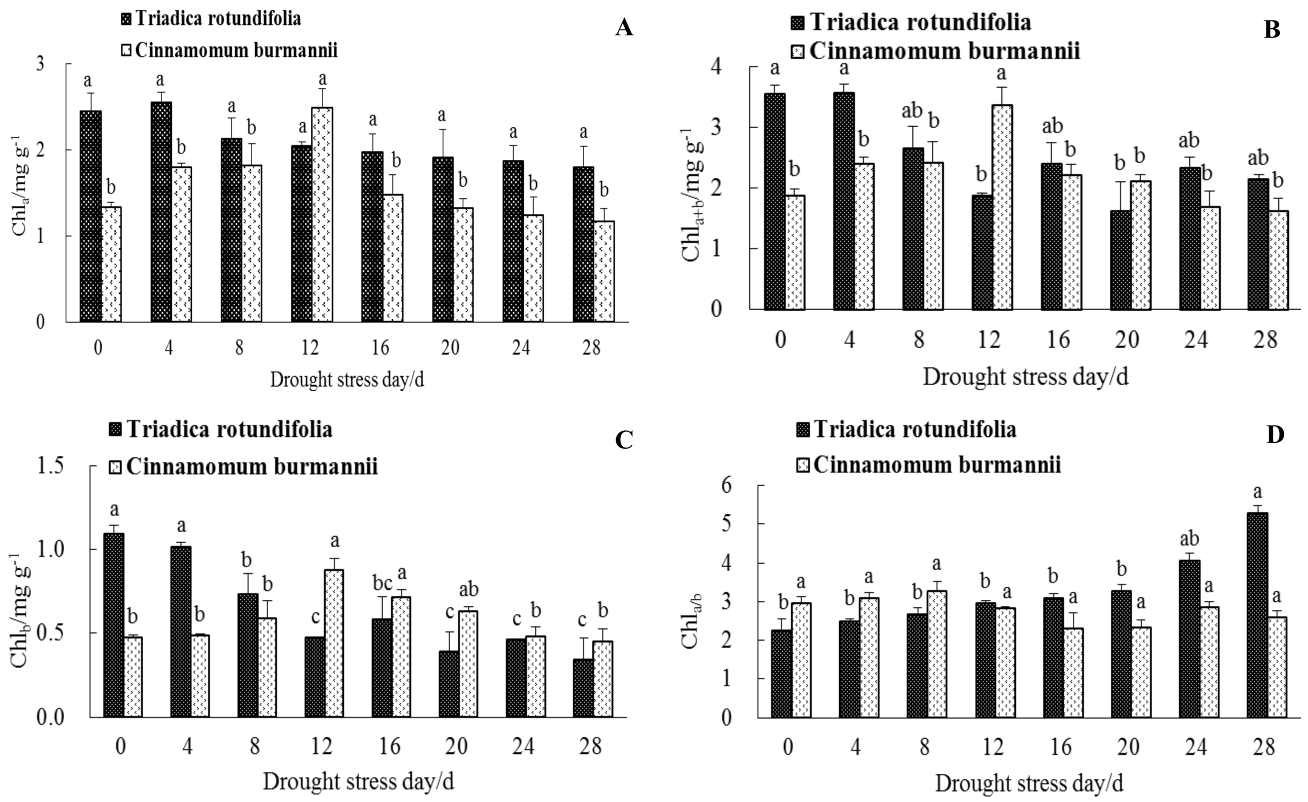

Fig. 1. Chlorophyll content comparison of two species in different drought periods Subplots A-D represent the comparison of *Chl*$_a$, *Chl*$_b$, *Chl*$_{a+b}$ and *Chl*$_{a/b}$, respectively. Mean values followed by the same letter within a column are not significantly different at the *p*=0.05 level for the soils at each species among drought treatments according to Tukey's HSD test.

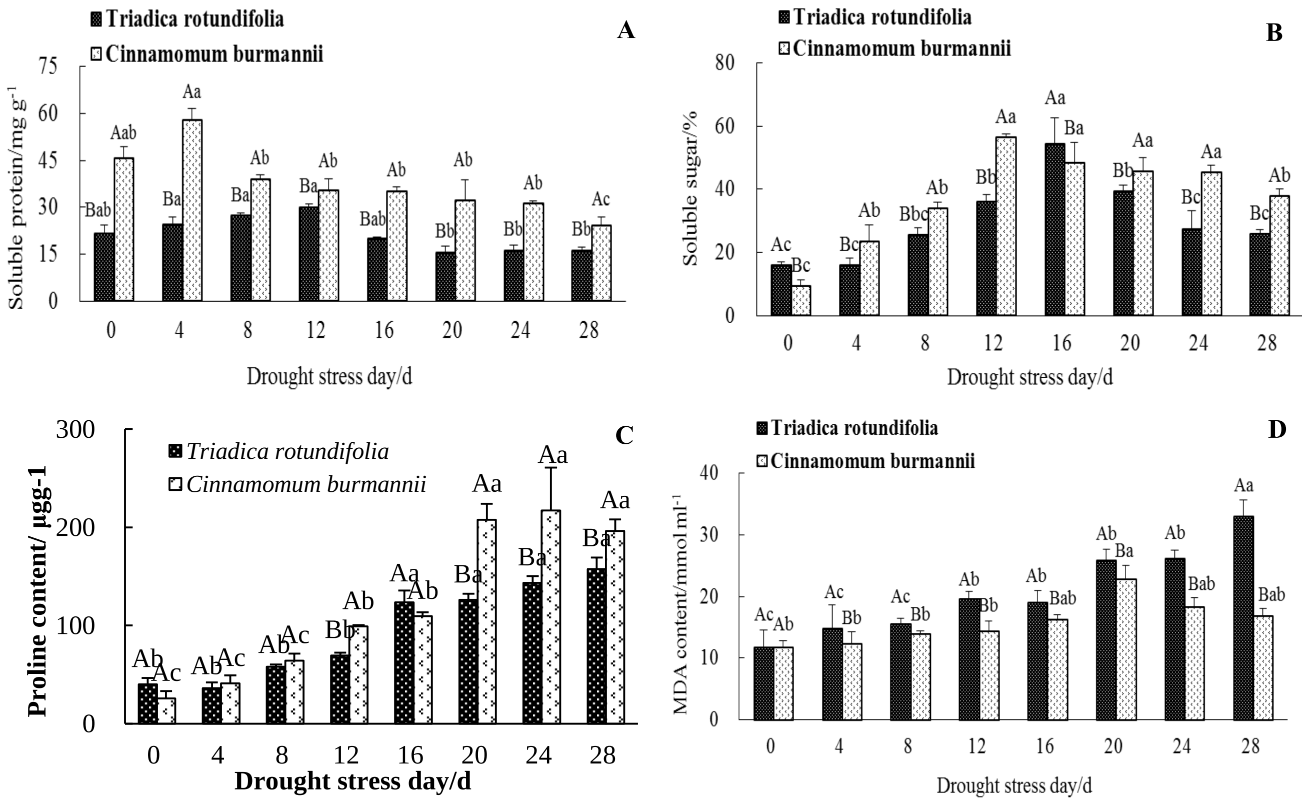

Fig. 2. Osmoticum and MDA contents of two species in different drought periods. Subplots represent soluble protein (A), soluble sugar (B), proline content (C), and MDA content (D). Means (at the top of each bar) with similar lowercase letters are not significantly different at $p<0.05$ level for the soils of the same species under different drought treatments, while those with the similar capital letters are not significantly different at $p<0.05$ level for different species under the same drought treatment, according to the multiple-comparison Tukey's HSD test.

Table 3. Changes in superoxide dismutase (SOD) and peroxidase (POD) contents of two species in different drought periods. Data are measured as the mean ± SE. SD values followed by the same letter (e.g., "a", "b", "c") in a column are not significantly different at the p=0.05 level by the multiple Tukey's HSD test. For example, *Triadica rotundifolia* had a non-significant difference on SOD content between day 0 and day 20.

Drought stress day/d	SOD		POD	
	Triadica rotundifolia	*Cinnamomum burmannii*	*Triadica rotundifolia*	*Cinnamomum burmannii*
0	143.64 ± 4.30c	174.22 ± 9.24b	84.15 ± 1.50a	1387.73 ± 18.80a
4	169.85 ± 9.15b	191.99 ± 1.10a	72.54 ± 8.18a	1488.14 ± 30.79a
8	174.11 ± 9.90b	191.25 ± 2.89a	60.29 ± 4.45a	966.43 ± 14.97b
12	190.84 ± 5.85a	190.62 ± 1.40a	67.81 ± 6.53a	853.17 ± 4.29b
16	174.35 ± 9.33b	183.41 ± 7.87b	45.92 ± 2.56b	852.48 ± 35.95b
20	154.94 ± 1.21c	168.29 ± 5.54b	37.13 ± 1.07b	650.57 ± 32.94c
24	139.69 ± 3.01c	124.85 ± 2.53c	22.23 ± 1.25c	601.86 ± 24.83c
28	127.87 ± 8.62c	118.29 ± 7.87c	19.72 ± 1.49c	406.11 ± 3.35c

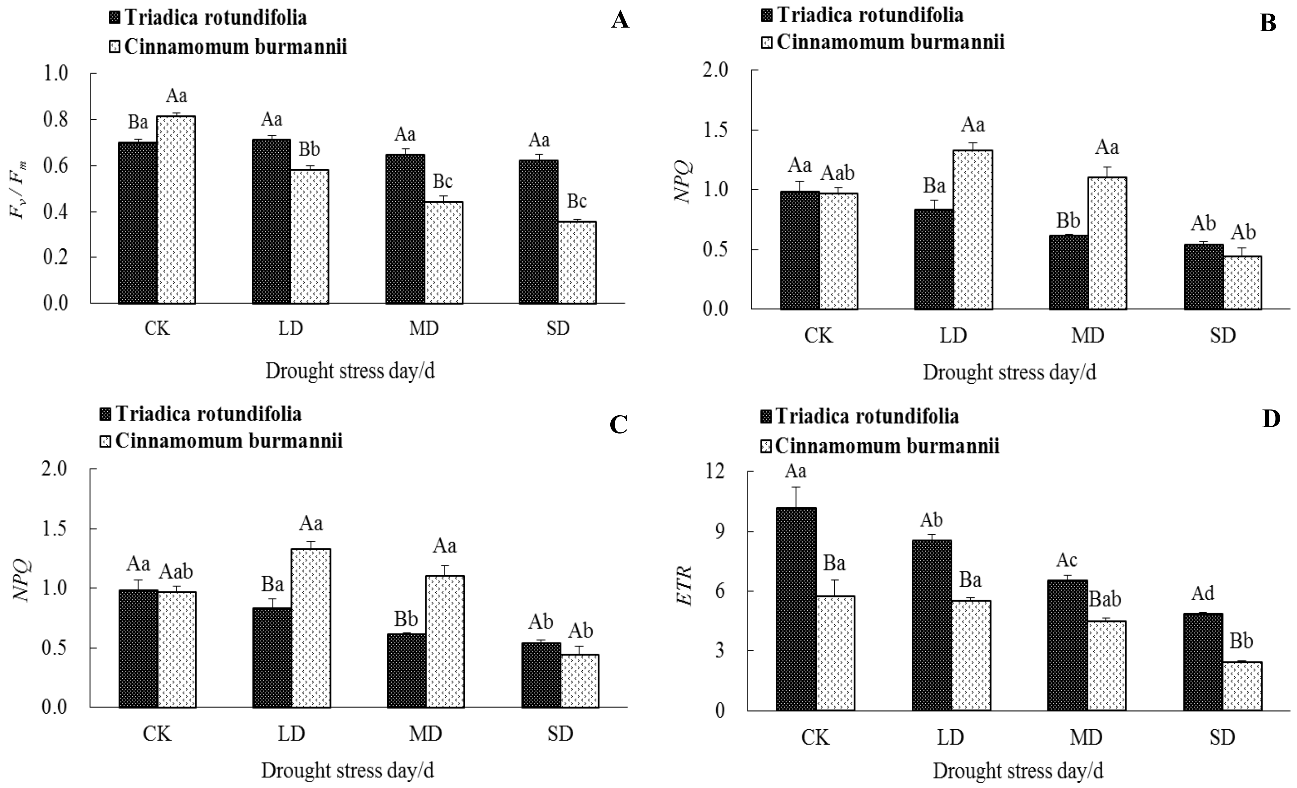

Fig. 3. Changing patterns of the maximum quantum efficiency of PSII photochemistry (F_v/F_m, in subplot A), electron transport rate (*ETR*, in subplot B) and nonphotochemical quenching (*NPQ*, in subplot C) of two species in different drought periods. Means (at the top of each bar) with similar lowercase letters are not significantly different at $p<0.05$ level for the soils of the same species under different drought treatments, while those with the similar capital letters are not significantly different at $p<0.05$ level for different species under the same drought treatment, according to the multiple-comparison Tukey's HSD test.

The values of *ETR* in the two species decreased as the drought stress was intensified. During the drought experiment, the *ETR* was significantly higher in *T. rotundifolia* than it was *C. burmannii*, and it decreased more sharply in *T. rotundifolia* over time (Fig. 3B).

The value of *NPQ* decreased significantly as the drought stress became intensified in *T. rotundifolia*, which showed an increasing trend at first and then a decrease under continuous drought stress in *C. burmannii* (Fig. 3C). Moreover, the *NPQ* maintained higher values in *C. burmannii* than in *T. rotundifolia* under the mild and moderate drought stresses.

Leaf anatomy: A cross-section analysis of the leaves of both species showed that both plants have a heterogeneous structure and are composed of an epidermis, leaf peduncle tissue and parameters (Fig. 4A-4D for *T. rotundifolia*; 4E-4H for *C. burmannii*).

The results of the combined ANOVA indicated a significant influence of drought stress on the studied anatomical leaf characteristics of the two species. In this case, drought stress resulted in a significant change in the leaf anatomical characteristics. The leaf thickness as well as the low epidermal, palisade parenchyma, spongy parenchyma and porosity of leaf tissues in *T. rotundifolia* were increased as the drought intensified (Table 4). With respect to the upper epidermal and the ratio of palisade to spongy parenchyma (P/S), significant decreases were estimated in the drought-stressed treatments (Table 4), whereas the tightness of leaf tissue and porosity of leaf tissue values in *T.*

rotundifolia showed a significant increase and then a decrease under the severe drought treatment (Table 4). In addition, this was more obvious in the structure in the epidermis papilla of *T. rotundifolia* (Fig. 4C, D).

Compared to the normal level treatment of *C. burmannii*, the leaf thickness and the upper epidermal, palisade parenchyma, spongy parenchyma, P/S ratio value, and porosity of leaf tissue (Table 4, Fig. 4E-H) were reduced under drought stress conditions. The leaf thickness value ranged from 295.58 μm to 365.08 μm, and the highest difference between treatments was found in the tightness of the leaf tissue. In the drought stress period, there was no distinct differentiation between palisade and spongy parenchyma, in which the cells had a disorderly arrangement (Fig. 4H).

PCA analysis on the physiological indices and leaf anatomy: The first principal component (PC1) accounted for more than 74.8% of the variance, and the second accounted for approximately 17.2% of the two species for all the treatments (Fig. 5). PC1 was accounted for mostly by the leaf thickness (TL), spongy parenchyma (TST) and P/S ratio value, while PC2 was accounted for by the *NPQ*, MDA and proline content. PC1 clearly separated the physiological indices and leaf anatomy from *T. rotundifolia* and *C. burmannii*. PC2 mainly separated the physiological indices and leaf anatomy from CL, LD, MD and SD. The treatments of *T. rotundifolia* are on the right, and the treatments of *C. burmannii* are on the left of the biplot (Fig. 5).

The biplot of PCA also showed that four groups could be identified: the CK of *T. rotundifolia*; LD of *T. rotundifolia*; MD and SD of *T. rotundifolia*; CK of *C. burmannii*; LD of *C. burmannii*; and MD and SD of *C. burmannii*. The r esults show that the distribution for the treatments of different phases among the two species was similar (Fig. 5).

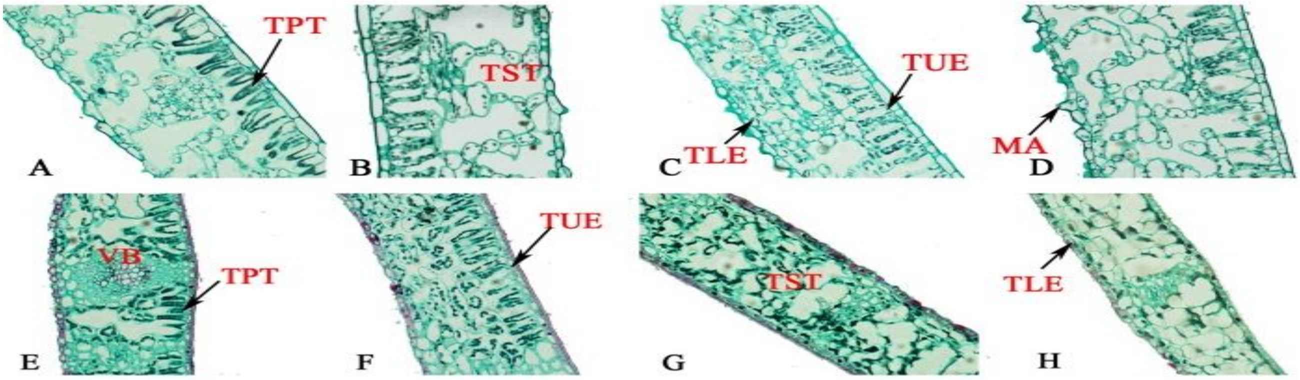

Fig. 4. Comparative cross-section leaf anatomy of the leaves in different drought treatments in two species. For the leaf anatomy of *T. rotundifolia*, a control treatment is represented by subplot (A), light drought stress (B), modern drought stress (C) and severe drought stress (D). For the leaf anatomy of *C. burmannii*, control treatment (E), light drought stress (F), and modern drought stress (G). ×200. Abbreviations: TUE: upper epidermal, TLE: lower epidermal, TPT: palisade parenchyma; TST: palisade parenchyma, VB: vascular tissue, MA: mammillae.

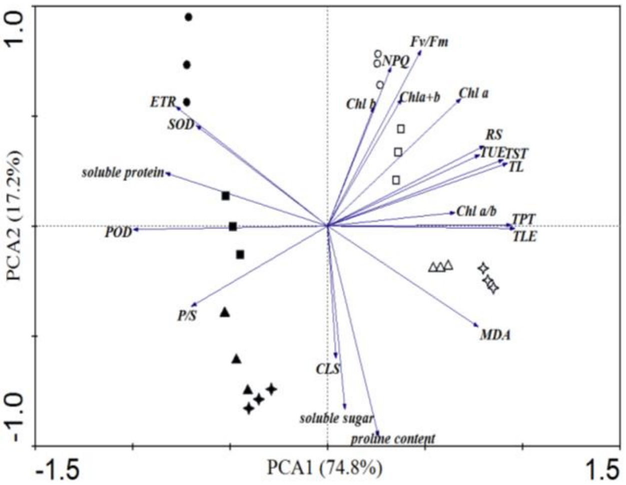

Fig. 5. PCA biplot for physiological indices and leaf anatomy for the two species in different treatments. Abbreviations: Chl_a: chlorophyll a content, Chl_b: chlorophyll b content, Chl_{a+b}: total chlorophyll content, $Chl_{a/b}$: the ratio of chlorophyll a/b, TL: leaf thickness, TUE: upper epidermal, TLE: lower epidermal, TPT: palisade parenchyma; TST: palisade parenchyma, P/S: the ratio of palisade/spongy, CLS: tightness of leaf tissue, RS: Porosity of leaf tissue.

Discussion

In general, the Chl_{a+b} content was affected by drought stress. In this study, the Chl_a, Chl_b, and Chl_{a+b} contents were affected to different degrees and decreased under severe drought stress. However, higher Chl_{a+b} and $Chl_{a/b}$ contents were found in *T. rotundifolia* than in *C. burmannii* in response to drought stress, suggesting that *T. rotundifolia* could contribute to a decrease in leaf water content (Souza *et al.*, 2004; Pan *et al.*, 2014).

The antioxidant enzyme activity and soluble compounds (soluble sugar, soluble protein, and proline) play a role in protecting and improving osmotic physiology against drought stress (Valliyodan & Nguyen, 2006; Li *et al.*, 2007; Zhu *et al.*, 2009; Conde *et al.*, 2011). In this study, the POD activity did not have different trends by drought stress treatments in the two species (Table 3), similar to the POD activity. The POD activity was more sensitive than the SOD activity in the two species, suggesting that the plants have evolved antioxidant enzymes, with POD being one of most important ones. Our observations indicate that regulating POD and SOD can mitigate the degree of drought stress, as supported by two previous studies (Pllrdy, 2010; Yao *et al.*, 2010).

Xu (2007) and Yao *et al*., (2010) found that the MDA content in *Acacia dealbata, Catalpa fargesii* and *Elaeocarpus decipiens* can be raised in response to drought stress. Our data are in accordance with this finding and confirm the increasing proline content and MDA content of *T. rotundifolia* (Fig. 2) under drought stress, suggesting that its lipid peroxidation resulted from oxidative stress and that it can adapt to be drought-resistant by enhancing its metabolism. In contrast, the proline and MDA contents of *C. burmannii* first increased and then significantly decreased as the drought stress intensified (Fig. 2). This response followed the same trend as that reported for *Croton lachnocarpus* (Hong *et al.*, 2015).

The parameters of chlorophyll fluorescence in two species, such as F_v/F_m, *ETR*, and *NPQ*, are related to photochemical processes. NPQ has been described as the most common form of protection against excessive light (Zhou *et al.,* 2007). In this study, under mild to severe drought conditions, the significant decreases in *ETR* and *NPQ* were coupled with the stable F_v/F_m in *T. rotundifolia* (Fig. 3), suggesting that PSII was a key photoprotection mechanism (Praxedes *et al.*, 2006) and that reducing the absorbance by decreasing pigment content is a photochemical mechanism in karst plants (Elsheery & Cao, 2008). It can remove excess energy to protect plants against fluorescence capture under drought stress. The decrease in F_v/F_m of *C. burmannii*, with a higher *NPQ* during moderate to severe stress, was associated with stable *ETR* under drought conditions (Fig. 3), which indicated that there was less excess energy of *C. burmannii* during photosynthesis. In accordance with previous studies of other species, such as *Pyracantha fortuneana* and *Pteroceltis tatarinowii*, the photosynthetic capacity declined under drought stress (Liu *et al.*, 2010). Furthermore, our results followed similar patterns reported in other studies (Souza *et al.*, 2004; Pan *et al.*, 2014).

Changes in leaf physiology and leaf anatomy are important for plants to be able to adapt to drought conditions. The leaf is the main tissue used for plant photosynthesis, and it includes the palisade parenchyma, spongy parenchyma, and cells of the palisade layer (Liu *et al.*, 2010). In this study, the strong drought-resistant capacity of *T. rotundifolia* may partially explain its dominance in the limestone region. Leaf traits, such as blade leaf thickness, upper and lower blade epidermis, strong leaf palisade and spongy parenchyma, are important determinants of the strong drought-resistant ability of the species (Table 4, Fig. 4). Different plants showed different sensitivity degrees to adaptation during stress (Rozendaal *et al.*, 2006). Zhang *et al*., (2000) found that spongy parenchyma has a strong intercellular space to reduce the burning of mesophyll cells. These results indicate that *T. rotundifolia* adjusts its leaf characteristics to adapt to drought stress. By contrast, the papilla in the epidermis suggests that these structures seem to be the predominant physiology mechanism of drought adaptation. Some similar observations of anatomical characters to adaptation under drought stress have also been reported (Deng *et al.*, 2004; Mo *et al.*, 2008; Zhang *et al.*, 2009). In contrast to the above, it has been suggested that plants with a spongy parenchyma that is closely arranged with small cell gaps sustains stress conditions (Zhang *et al.*, 2000).

The anatomical data suggest that *C. burmannii* is more productive under normal and light drought conditions but has lower drought tolerance under severe drought stress. The confusion is between the palisade and spongy parenchyma and the thin leaf thickness and cuticle (Table 4, Fig. 4). These results contrast with reports in the literature showing that an increase in the structure associated with leaf water transport may be considered drought stress. These characteristics indicated that the leaf structure of *C. burmannii* could adapt well to light drought stress, as also found by Tang *et al*., (2012).

Plants can adjust their leaf morphology, structure, and physiology to accommodate variations in stress (Pinto-Marijuan & Munne-Bosch, 2013; Yang *et al.*, 2014; Rajpar *et al.*, 2018). In this study, we found physiological and anatomical differences between *T. rotundifolia* and *C. burmannii* in response to drought stress. The drought tolerance of *T. rotundifolia* was higher than that of *C. burmannii* under various drought stresses.

Acknowledgements

This research was funded by the Science and Technology Planning Project of Guangdong Province, China (No. 2015A020209131) and the Innovation of Forestry Science and Technology Foundation of Guangdong, China (No. 2008KJCX010-3, 2012KJCX014-01, 2015KJCX021).

References

Catoni, R., L. Gratani and L. Varone. 2012. Physiological, morphological and anatomical trait variations between winter and summer leaves if Cistus species. *Flora,* 207: 442-449.

Chen, J. and X. Wang. 2002. Experimental instruction of plant physiology. South China University of Technology Press, Guangzhou, China.

Clements, R., N. Soddhi, M. Schilthuizen and K. Peter. 2006. Limestone karsts of Southeast Asia: Imperiled arks of biodiversity. *Bioscience,* 56: 733-742.

Conde, A., M. Manuela Chaves and H. Geros. 2011. Membrane transport, sensing and signaling in plant adaptation to environmental stress. *Plant Cell Physiol.,* 52: 1583-1602.

Deng, Y., Z. Jiang, J. Cao, Q. Li and F. Lan. 2004. Characteristics comparison of the leaf anatomy of Cyclobalanopsis glauca and its adaption to the environment of typical karst peak cluster areas in Nongla. *Guihaia,* 24: 317-322.

Dong, L., H. Cao, W. Ye, Z. Xu, L. Wu and Y. Chen. 2011. Leaf anatomic traits of 5 species in southwestern China's karst region. *Chinese J. Appl. Environ. Biol.,* 17: 747-749.

Elsheery, N. and K. Cao. 2008. Gas exchange, chlorophyll fluorescence, and osmotic adjustment in two mango cultivars under drought stress. *Acta Physiol. Plant.,* 30: 769-777.

Fu, S. and B. Huang. 2004. Analysis on succession trend and physiological characters of secondary forest tree species in limestone mountain of eastern Anhui province. *Sci. Silvae Sin.,* 24: 317-322.

Han, G. and Z. Zhao. 2010. Light response characteristics of photosynthesis of four xerophilous shrubs under different soil moistures. *Acta Ecol. Sin.,* 30: 4019-4026.

Hong, W., R. Xu, Y. Wei, M. He and X. Zhuang. 2015. Seedling growth, morphology and physiological characteristics of Triadica rotundifolia and Croton lachnocarpus under drought stress. *J. South China Agric. Univ. (Nature Sci. Ed.,* 36: 79-84.

Huang, S., Y. Pan, D. Gan, X. Ouyang, S. Tang, S. Ekunwen and H. Wang. 2011. Antioxidant activities and UV protective properties of melanin from the berry of Cinnamomum burmannii and Osmanthus fragrans. *Median. Chem. Res.,* 20: 475-481.

Li, Y., L. Xue and M. Wu. 2007. Research advances in mechanisms tree species drought resistance. *Chinese J. Ecol.,* 26: 1857-1866.

Liu, C., Y. Liu, K. Guo, Y. Zheng, G. Li and L. Yu. 2010. Influence of drought intensity on the response of six woody species subjected to successive cycles of drought and rewatering. *Physiol. Plant.,* 139: 39-54.

Maxwell, K. and G. Johnson. 2000. Chlorophyll fluorescence-a practical guide. *J. Exp. Biol.,* 51: 659-668.

Mo, L., Y. Huang, J. Qin, X. Wang, S. Lu and W. Yuan. 2008. A preliminary study on the water physiology of four plant species in karst area of southwest China. *Guihaia,* 28: 402-406.

Nardini, A., G. Peda and N. La Rocca. 2012. Trade-offs between leaf hydraulic capacity and drought vulnerability: morpho-anatomical bases, carbon costs and ecological consequences. *New Phytol.,* 196: 788-798.

Pan, X., Q. Qin, J. Li, J. Wang, Q. He, Y. Su, J. Ma and K. Du. 2014. Physiological indexes of six plant species from the tibetan plateau under drought stress. *Acta Ecol. Sin.,* 34: 3558-3567.

Pinto-Marijuan, M. and S. Munne-Bosch. 2013. Ecophysiology of invasive plants: osmotic adjustment and antioxidants. *Trends Plant Sci.,* 18: 660-669.

Pllrdy, S. 2010. Physiology of woody plants. Academic Press, New York.

Praxedes, S., F. DaMatta, M. Loureiro, M. Ferrao and A. Cordeiro. 2006. Effects of long-term soil drought on photosynthesis and carbohydrate metabolism in mature robusta coffee (Coffea canephora Pierre var. kouillou) leaves. *Environ. Exp. Bot.,* 56: 263-273.

Rajpar, I., M.S. Lashari, S.K. Brahman, M. Ali, Z. Shah, B.N. Mangan and A.H. Shah. 2018. Salinity and drought stress tolerance of some plant species of Thar Desert, Pakistan: implications in bio-saline agriculture. *Pak. J. Bot.,* 50: 465-469.

Ren, H. 2005. A review on the study of desertification process and restoration mechanism of karst rocky ecosystem. *Trop. Geogr.,* 25: 195-200.

Rong, L., S. Wang, G. Yu, X. Deng and J. Ran. 2012. Stable isotope analysis of water sources of four woody species in the Libo karst forest. *Sci. Silvae Sin.,* 48: 14-22.

Rozendaal, D., V. Hurtado and L. Poorter. 2006. Plasticity in leaf traits of 38 tropical tree species in response to light; relationships with light demand and adult stature. *Funct. Ecol.,* 20: 207-216.

Souza, R., E. Machado, J. Silva, A. Lagoa and J. Silveira. 2004. Photosynthetic gas exchange, chlorophyll fluorescence and some associated metabolic changes in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environ. Exp. Bot.,* 51: 45-56.

Tan, M., D. Gan, L. Wei, Y. Pan, S. Tang and H. Wang. 2011. Isolation and characterization of pigment from Cinnamomum burmanniipeel. *Food Res. Int.,* 44: 2289-2294.

Tang, W., S. Chen and J. Weng. 2012. Leaf anatomic structure of Cinnamomum burmannii. *Guangdong Agric. Sci.,* 39: 43-45.

Valliyodan, B. and H. Nguyen. 2006. Understanding regulatory networks and engineering for enhanced drought tolerance in plants. *Curr. Opin. Plant Biol.,* 9: 189-195.

Wu, J., F. Qin, W. Wang, P. Chen, S. Gao, H. Xu, Q. Lin, C. Zhu and Y. Yu. 2010. Research progress of soil and peculiar plants in limestone areas of China. *Jiangsu For. Sci. Technol.,* 37: 50-54.

Wu, Z. 2004. Flora of China (Vol. 31). Science Press, Beijing, China.

Xu, L. 2007. Study on afforestation tree species seedlings with drought resistance in rocky desertification area. Southeast University.

Xu, L., C. Carrie, S. Law, M. Murcha and J. Whelan. 2013. Acquisition, conservation, and loss of dual-targeted proteins in land plants. *Plant Physiol.,* 161: 644-662.

Yang, S., M. Sun, Y. Zhang, H. Cochard and K. Cao. 2014. Strong leaf morphological, anatomical, and physiological responses of a subtropical woody bamboo (*Sinarundinaria nitida*) to contrasting light environments. *Plant Ecol.,* 215: 97-109.

Yao, H., J. Xue, Q. Wu, Y. Wu and Y. Rong. 2010. Drought resistance of four species of tree saplings for afforestation in karst regions. *J. Ecol. Rural Environ.,* 26: 318-322.

Zhang, J., J. Li and G. Shen. 2000. Study on drought resistance characteristics and mechanism of trees. China Forestry Press, Beijing, China.

Zhang, Z., Y. Huang, L. Mo and W. Yuan. 2009. Photosynthesis light response characteristics of four limestone plants in karst area. *J. Northwest For. Univ.,* 24: 44-48.

Zhou, X., H. Zhao and C. Ma. 2007. Effects of silicon on chlorophyll fluorescence of cucumber seedlings under severe drought stress. *Acta Agric. Boreali-Sinica,* 22: 79-81.

Zhu, H., H. Wang, B. Li and P. Sirirugsa. 2003. Biogeography and floristic affinities of the limestone flora in southern Yunnan, China. *Ann. Missouri Bot. Gard.,* 90: 444-465.

Zhu, J., J. Zhang, H. Liu and K. Cao. 2009. Photosynthesis, non-photochemical pathways and activities of antioxidant enzymes in a resilient evergreen oak under different climatic conditions from a valley-savanna in Southwest China. *Physiol. Plant.,* 135: 62-72.

(Received for publication 19 August 2017)