Adaptability Comparison between the Seedlings of *Eucalyptus grandis* and *Alnus cremastogyne* under the Condition of Continuous Drought Stress

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Abstract

A pot experiment was carried out to study the growth and physiology responses of *Eucalyptus grandis* and *Alnus cremastogyne* seedlings subjected to continuous drought stress. The results were as follows (1) In the case of short treatment time (0~6 d), the leaf water status of both species were good, but comparatively higher stomatal conductance(Gs) and transpiration rate(Tr) values of *E. grandis* leaves might lead to higher water consumption. (2) In the more severe drought conditions(9~18 d), both species could avoid drought threat by decreasing Gs and Tr. However, *A. cremastogyne* also took the strategy of defoliation so that the remaining leaves could get adequate water, resulting in its better leaf water status and slighter responses of osmoregulation substances and antioxidant enzymes during 18 d treatment. (3) *A. cremastogyne* was more sensitive to drought than *E. grandis* because of the earlier significant rises but unstable changes of the activity of superoxide dismutase(SOD) and the contents of H_2O_2, soluble protein(SP) and proline(Pro) in leaves. (4) In face of drought stress, *A. cremastogyne* might mainly focus on the drought-avoid strategy to save water and reduce oxidative damage at the cost of growth, however, *E. grandis* consumed more water for assimilation and production, probably evolved out of a relatively stronger mechanism to face the drought threat such as oxidation damage.

Keywords: *Eucalyptus grandis*, *Alnus cremastogyne*, Continuous drought, Physiological response, Adaptive strategy

1. Introduction

Water is one of the key factors which affect the growth, distribution and introduction of plants (Jia et al, 2002; Shvaleva et al, 2005). It is well known that about one third of the continent is in arid and semi-arid status (Guo et al, 2003; Luo & Li, 2005). And more seriously, seasonal or periodic droughts also happened all over the world occasionally for the complexity of the regional geographic and meteorological factors and the impact of global climate change in recent years (Wang et al, 2008;Yao et al, 2009; Yang, 2008). During the dry season (from Jan. to Mar.), as Zhong et al (2001) observed in eucalyptus stands of Leizhou Peninsula, Guangdong, China, soil water content at the depth of 1~40 cm was nearly wilting point. If there were not rain for several days, soil water content at 0~80 cm dropped to about 30% of the field capacity. The growth of crops and trees must be affected to some extent in this case and thus selecting drought-resistant tree species and carrying out rational water management become extremely critical.

Eucalyptus grandis, with the features of evergreen, strongly positive, fast-growing, good stem form and

adaptable (Feng & Zhang, 2005; Ni et al, 2007), has been developed rapidly throughout the world, especially in India, Brazil and China (Feng et al, 2008). *Alnus cremastogyne,* deciduous, thermophilic, heliophilous, poor soil tolerating, non-leguminosae nitrogen-fixing plant with rhizobium on root (Wang et al, 2001;Liang et al, 2010), has the potential to be very promising broad-leaved tree species for commercial purposes and vegetation restoration in the south of the Yangtze River (Zhu et al, 2004a). Both are widely used, and play important roles in the projects of short-cycle industrial timber bases.

With regard to the physiology and growth response to drought and adaptation strategies of *Eucalyptus* and *Alnus* species, there have been numerous reports, such as the response of leaf water status and the morphology of *E. globulus* (Pita & Pardos, 2001), the osmolytes of *E. globulus* and *E. astringens* (Shvaleva et al, 2005; Ardnt et al, 2008), the photosynthetic parameters of *E. globulus* and *E. camaldulensis* to water deficit (Gindaba et al, 2004; Zhong et al, 2003), and the influence of drought on the xylem function of *A. glutinosa* (Hacke & Sauter, 1996), the water relations, photosynthetic rate and water use efficiency of *A. firma* and *A. formosana* (Liang & Maruyama, 1995; Zhu et al, 2004 a, b), etc. These studies tended to merely focus on one side of growth and physiology, and the comparisons were mostly made between clones or congeneric species, but very few integrated comparisons on the drought adaptability, especially between *E. grandis* and *A. cremastogyne*, have been reported. Additionally, the analysis and evaluations based on the water status, osmotic adjustment, antioxidant metabolism and photosynthesis under the same experimental condition have been rarely found in publications.

In terms of the experimental designs, the formers often took the approaches which controlled soil moisture level in the series (Pita & Pardos, 2001; Guo et al, 2003; Zhong et al, 2003; Shariat & Assareh, 2008), even the method of PEG stress simulation was used (Zhu et al, 2004b). Obviously it was difficult to reflect the response of the trees which suffered from the continuous drought in the field by the studies mentioned above. Therefore, this study, with pot experiment and continuous drought stress for different days, simulated prolonged drought conditions experienced by *E.grandis* and *A. cremastogyne* to observe their growth and physiology response, analyzed and compared their drought-resistant (drought-tolerant) characteristics and adaptation mechanisms to provide a theoretical reference for the selection of afforestation tree species and water management in the periodic drought-prone areas.

2. Materials and Methods

2.1 Site Description

The experiment was conducted at the teaching and research farm of Sichuan Agricultural University, southwest of Sichuan Province, China (29°58′ 48″ N, 102°59′ 55″ E, 600 m altitude, a. s. l.).The annual average temperature is 14.1 ℃ ~ 17.9 ℃, ≥ 10 ℃ accumulated temperature is 5231 ℃, monthly mean maximum temperature 29.9 ℃ (Jul.), monthly average minimum temperature 3.7 ℃ (Jan.). Average annual sunshine hours 1039.6 h, frost-free period 298 d, the average annual rainfall 1774.3 mm, average air humidity of 79%, subtropical humid monsoon climate with four distinct seasons, rains are more concentrated in the summer, a small temperature difference between day and night, much rain, insufficient light.

2.2 Soil and Tree Seedlings

The local common sandy loam, with the field capacity of 25% (volumetric water content) and the bulk density of 1.32 g • cm^{-3}, disinfected with carbendazim, thoroughly mixed and then maintained ventilation for 2 d. The tissue culture plantlets of *E. grandis* and the normal seedlings of *A. cremastogyne* were cultivated in 2010 spring and winter, respectively, with the average height of 17.5 cm and 30.0 cm. Both were planted in the plastic bucket loaded with 15 kg of soil, 1 seedling per bucket. HH2 soil moisture analyzer (ML2x, GBR) was used to monitor and control the soil water content at about 20% (about 80% of field capacity) in the bucket. Adaptive training of young trees were performed for 5 months before treatments.

2.3 Experimental Treatments

Uniform seedlings (21 stems for each) of two species were selected on October 9, 2010 and then both divided into 7 groups (3 stems for each group). After the last normal watering, 3 d as a treatment period, stopped watering for each group successively until October 27, then a drought time series of 0, 3, 6, 9, 12, 15, 18 d with soil moisture gradient formed, denoted as D0 (CK), D3, D6, D9, D12, D15, D18 (Tab. 1), respectively.

2.4 Measurement Items and Methods

In the end of drought stress (October 28, 2010), the soil water contents in all buckets were measured, and leaf samples were taken for the determination of morphological and physiological indicators.

2.4.1 Height and Surface Diameter Growth

Height and diameter increment: the initial value of height (H_1) and surface diameter (S_1) were measured at the beginning of drought stress (October 9, 2010), and measured again in the end of drought stress (October 28, 2010), denoted as H_2 and S_2, respectively. Height increment $\triangle H = H_2 - H_1$, and diameter increment $\triangle S = S_2 - S_1$.

2.4.2 Water Physiological Traits

Water physiological indices were determined with the Reference of Whetherley (1950), Gindaba et al (2004). The mature leaves in the same position of upper parts of plants were cut and immediately weighed to get the fresh weight (FW), then soaked in distilled water until no further increase in their weight, which at this time was called saturated weight (TW). After that, took out for natural drying indoors for 24 h and weighed for the FW ', then water-removed in the oven at 105 ℃ for 30 min, finally dried to constant weight (DW) under the conditions of 80 ℃. Results of calculations were as follows:

Leaf water content (LWC) = (FW-DW) / FW × 100%

Leaf relative water content (LRWC) = (FW-DW) / (TW-DW) × 100%

Leaf water retention capacity (LWRC) = [1 - (TW-FW ') / (TW-DW)] × 100%

2.4.3 Photosynthesis and Gas Exchange Parameters

Using LI-6400 portable photosynthesis meter (LI-COR Inc.USA) to determine the net photosynthetic rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr). Instrument parameters were set as: light intensity 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, CO_2 concentration 400 $\mu mol \cdot mol^{-1}$, temperature 25 ℃. Three replications for each treatment, and five readings for each replication. Water use efficiency (WUE) was calculated according to the formula WUE = Pn / Tr (Penuelas et al (1998)).

2.4.4 Indicators Related to Osmotic Adjustment

Malondialdehyde (MDA) and soluble sugar (SS) determination: with 10% trichloroacetic acid (TCA) as grinding solution, 0.6% thiobarbituric acid (TBA) as the chromogenic agent, the extraction was colored in 100 ℃ water bath for 15 min; Soluble protein (SP) determination: with 0.05 mol / L pH7.0 phosphate buffer as grinding solution, then the supernatant reacted with Coomassie brilliant blue G250 solution. Free proline (Pro) was extracted by 3% 5 - sulfosalicylic acid in boiling water bath for 15 min, colored by ninhydrin reaction in boiling water bath for 30 min, extracted with toluene when cooling naturally, and finally the toluene solution was colorimetric analyzed; The parameters above were all measured by the spectrophotometry suggested by Xiong (2003).

2.4.5 Indicators Related to Reactive Oxygen Metabolism

Hydrogen peroxide (H_2O_2) content was measured with reference to the method suggested by Lin et al (1988) : According to the principle that H_2O_2 and titanium ion bind to colored $[TiO (H_2O_2)]^{2+}$, H_2O_2 was determined by the colorimetry ; Superoxide dismutase (SOD) activity was determined using the method called NBT photochemical reduction, 50% activity of SOD photochemical reduced was calculated as a unit U of activity (Giannopolitis, 1977); Peroxidase (POD) activity was determined by the method of guaiacol (Xiong, 2003), the increment of 0.01 at A_{470nm} per min was defined as a unit U of activity; Ascorbic acid (AsA) content was determined in the light of Hodges et al (1996). The reaction of AsA and ammonium molybdate in the presence of sulfate and phosphate ions generated a blue complex, then AsA content was determined by the colorimetry.

2.5 Statistical Analysis

One-way ANOVA with Fisher's LSD test and Person linear correlation analysis were conducted using SPSS 16.0 (SPSS Inc., USA) statistical analysis software for windows. Mapping was performed using Microsoft Excel 2003. Significant effects were determined at $P < 0.05$ for significant difference or $P < 0.01$ for extremely significant difference. Mean values in the text were given ± 1 SE.

3. Results

3.1 Response of Leaf Water Status to Continuous Drought

The water content of soil used to cultivate the two species both declined with the extension of drought time and showed the trend of "slow - fast - slow", and the water content of soil on which *A. cremastogyne* seedlings were planted was obviously higher than that of soil planted with *E. grandis* seedlings, but after 12 d of drought this difference decreased (Fig. 1 D). The leaf water content (LWC) of *E. grandis* remained stable during 0 ~ 6 d, while relative water content (LRWC) was significantly reduced after 6 d. With the aggravation of drought stress, both LWC and LRWC declined rapidly, amplitudes were even up to 49.83% and 61.77 %, respectively, when

drought for 18 d. But the fluctuation of LWC of *A. cremastogyne* was small (in the range of 5.76%) during the whole drought period of 18 d, and the drop of LRWC was only 1.40% ~14.82% (Fig. 1 A, B). Correlation analysis showed significant positive correlation between the LWC and soil moisture content, and extremely significant positive correlation between the LRWC and soil moisture content referred to *E. grandis*. For *A. cremastogyne,* only LRWC is significantly positive correlated to soil moisture, indicating that the leaf water status of *A. cremastogyne* is relatively stable in the dry process. From fig. 1 C, we can see that the change of leaf water retention capacity (LWRC) of the two species showed the opposite trends, and the LWRC of *E. grandis* was always clearly higher than that of *A. cremastogyne.*

3.2 Response of Leaf Osmolytes to Continuous Drought

In the process of drought stress, free proline (Pro), soluble protein (SP), soluble sugar (SS) and other osmolytes in the plants play important roles in the maintenance of cell turgor and the protection of proteins, enzymes and membranes. The changes of these substances reflect the dehydration tolerance of receptors (Blum, 1996; Li et al, 1996; Smirnoff, 1998). In the first 9 d of drought, the Pro content in leaves of *E. grandis* showed slight changes, then remarkably increased, and were approximately 7, 4 and 22 times that of normal water supply conditions when drought stressed for 12, 15, 18 d, respectively. Also for the SP content in leaves of *E. grandis* there were no significant differences between D0 and D3 ~ D9, but it increased significantly or extremely significantly with the treatments D12 ~ D18. The response of SS was faster. Under D3, the change of SS was found mild, but enhanced significantly or extremely significantly under D6 ~ D18 (Fig. 2 A, B, C); The Pro content in *A. cremastogyne* significantly increased under treatment D6, and gradually rose until D12, then began to decrease but still significantly higher than that of D0. The highest increase was about 3 times that of D0. SP increased first in treatment D3 and D6 and then fell to the lowest in D15. A recovery was seen in D18 but still below that of D0. SS did not change significantly during first 9 d of drought stress, but remarkably reduced when treated for 12 d. Obviously, *A. cremastogyne* was more sensitive to drought, and moreover, the response of osmolytes in its leaves was neither strong nor positive compared to that of *E. grandis* (Fig. 2 A, B, C).

3.3 Response of Leaf Antioxidant System to Continuous Drought

Scavenging reactive oxygen species (ROS) depends on the members of the enzymatic defense system (superoxide dismutase (SOD), peroxidase (POD), etc.) and the secondary metabolites (β-carotene, ascorbic acid (AsA), etc.). Their contents or activities can describe to some extent the resistance capability of plants to drought (García-Plazaola et al, 1997; Sgherri et al, 2000; Wang et al, 1989). With the increasing threat of drought, the SOD and POD activity of *E. grandis* leaves increased gradually but slightly if the drought time was less than 6 d. After 9 d, they were significantly higher and the trend of both appeared to be flat. The SOD activity and the POD activity were positively and significantly correlated and both increased significantly together with H_2O_2; AsA content of the treatment of 18 d significantly increased, while that of other treatments had no significant difference from D0 or even lower than D0 instead; As for *A. cremastogyne*, The SOD activity was promoted extremely significantly when treated for 3 d, decreased gradually after that, and began to rebound at 12 d, reached the highest at 18 d. The activity of POD first increased, then decreased with the extension of drought, the peak value appeared at D9, and the values lower than that of D0 revealed after water deficit for 15~18 d. In contrast to SOD activity, AsA content decreased first, increased then and decreased again, showed an obvious fluctuation but was significantly lower than that of D0 under all stress treatments (Fig. 3 A, B, C). This indicated that in face of the threat of drought, both species were able to mobilize internal enzymatic removal system actively to prevent the damage caused by ROS, however, *E. grandis* could maintain the system at a high level while *A. cremastogyne* could not. AsA of both species played a minor role as antioxidant.

3.4 Response of Leaf H_2O_2, MDA to Continuous Drought

Drought has the potential to induce ROS, such as superoxide anion radical (O_2^-) and hydrogen peroxide (H_2O_2), which implement the function of signal transduction but generate oxidative stress as well when the concentrations of which are high enough (Smirnoff, 1998; Finkel et al, 1998; Li, 2006) and can be diagnosed by malondialdehyde (MDA) and other lipid peroxidation products (Rubio et al, 2002; Chen et al, 2000). It could be seen from Fig. 4 and 5 that H_2O_2 in *E. grandis* leaves increased significantly (except D3), and presented a rising trend with the extension of drought time. Meanwhile, MDA content increased constantly, and significantly positively correlated with H_2O_2 content, indicating that *E. grandis* leaves under drought stress were facing more serious threats of ROS and oxidative damage. While the content of H_2O_2 in *A. cremastogyne* leaves showed an apparent fluctuation with the extension of drought time and there was no specific regulation. The content of MDA was not significantly changed during the course of 18 d of drought, implying that *A. cremastogyne* leaves might suffer less oxidative threat.

3.5 Response of Photosynthetic Parameters to Continuous Drought

Fig. 6 ~ 8 showed that in a certain range of treatment time (0 ~ 6 d), the stomatal conductance (Gs) and transpiration rate (Tr) of *E. grandis* leaves maintained at a high level, the net photosynthetic rate (Pn) was not significantly reduced either. Nevertheless, the 3 parameters above declined sharply and remained at a very low level probably for the aggravation of water deficit after 9 d of drought. The Gs, Tr, Pn of *A. cremastogyne* leaves increased significantly in D6 and were obviously lower than those of *E. grandis* during D0 ~ D6, however, the difference of which between *A. cremastogyne* and *E. grandis* reduced when drought intensified. As to water use efficiency (WUE), *E. grandis* leaves developed stably in the initial drought (D0 ~ D6), but remarkably increased when drought for 9 d, and declined then. The leaf WUE of *A. cremastogyne* had a similar trend with but higher than that of *E. grandis*, the maximum of which was seen in D12, since then maintained at a high level (Fig. 9). The correlation analysis indicated that the Pn, Gs, Tr of *E. grandis* and its soil moisture were all significantly and positively correlated, while there were no significant linear relationships between the 3 parameters of *A. cremastogyne* and its soil moisture.

3.6 Response of Surface Diameter and Height Growth to Continuous Drought

Tab. 2 showed that, with the drought time extended, the height growth of *E. grandis* was gradually inhibited. While for *A. cremastogyne*, which presented the uptrend first and downtrend then, even was in D6 significantly higher than that of D0, but negative height growth appeared in D15 and D18 due to the tip wilting of the seedling caused by severe drought. In general, the height growth of *E. grandis* was higher than that of *A. cremastogyne* to a large extent, and in severe drought (12 ~ 18 d) the fall of growth of *E. grandis* was smaller than that of *A. cremastogyne*. Besides, the increment of surface diameter of *E. grandis* was basically more than that of *A. cremastogyne* during the process of 18 d drought, and the diameter increment of both species were negative when drought for 18 d probably for the contraction of the xylem caused by serious water deficit.

4. Discussion

The response of plants to drought stress can be three ways -- escape, avoidance and tolerance (Li, 1998; Chaves et al, 2002). Drought escape means that the plant will complete its life history before the advent of drought. Annuals mainly adopt this way, but clearly impossible for *A. cremastogyn*e and *E. grandis*; Drought avoidance refers to that some plants prevent water loss or enhance water absorption capacity by reducing leaf area index (Gibson et al, 1991; Lauteri et al, 1997), leaf area (Teklehaimanot et al, 1998), forming well-developed palisade tissue (Ma et al, 2002) and thick cuticle (Li, 1998; Luo & Li, 2005) and changing biomass allocation (Silva et al, 2004), etc. Some plants increase the concentrations of intracellular osmoregulation substances (Delauney & Verma, 1993) and regulate the activities of antioxidant enzymes (Rubio et al, 2002), etc., to deal with dehydration caused by drought stress and avoid further damage, which is defined as drought tolerance. Plants (even intraspecies) living in different environments for a long time have developed various ways and means to adapt to different conditions (Pita & Pardos, 2001).

4.1 Water Consumption and the Strategies for Avoiding Drought of E. grandis and A. cremastogyne

An et al (1993) found that the vascular area of *E. grandis* was small, the structure of which was simple and the palisade tissue was thin and loose, therefore the conclusion they reached was that *E. grandis* could grow better in the soil with good moisture condition. By contrast, well-developed palisade tissue maybe was one of the characteristics of drought resistance of *A. cremastogyne* as Liang et al (2010) reported. However, the results of this study supported the point that leaves of *E. grandis* possessed stronger anti-dehydration than those of *A. cremastogyne* in terms of the water retention capacity, which was in accord with the reports on *E. grandis* given by Clarke & Romagosa (1989) and Sun et al (2009). Both species decreased their stomatal conductance to reduce transpiration when aridity reached a certain level. The reason might be that the stress induced the synthesis of a large number of abscisic acid(ABA) in the root , which was transported to the upper parts of aboveground, thus led to stomatal closure (Li et al, 2004). Compared with *A. cremastogyne*, the stomatal conductance and transpiration *of E. grandis* leaves were obviously higher under the condition of normal water supply or at the beginning of drought, and the leaf water content dropped more rapidly with the decline of soil moisture, indicating a larger amount of water consumption. This might be related to the different strategies the two species used to avoid drought. Li (1998) and Li & Zhu (2006) have reported that in the dry season or under drought stress plants decreased or prevented the water loss by leaf defoliation, wilting, curling or producing smaller blades. In fact, Acute defoliation was observed at the lower and middle part of the plant in the process of drought to *A. cremastogyne*, while *E. grandis* rarely defoliated even completely withered in the later period of drought. This was similar to the result obtained by Gindaba et al (2004) that three deciduous tree species retained only the latest germinated leaves, thus the water status of which was better than that of two *Eucalyptus* under

drought stress. As a result, comparatively larger total leaf area and total transpiration but easier water deficit happened to *E. grandis*.

4.2 The Physiological-biochemical Regulation and Drought-tolerant Strategy of E. grandis and A. cremastogyne

It was inevitable that leaves of *E. grandis* suffered dehydration during the drought because of its non-defoliation strategy coupled with strong transpiration in the early stage of drought, so the drought-tolerant mechanisms had to be enabled. On the one hand, SOD, POD activity rose collaboratively to cope with the accumulation of drought-induced ROS, such as O_2^- and H_2O_2, so as to prevent oxidative damage, whereas such ability was limited. In the short term drought (0 ~ 6 d, the drop of soil moisture was less than 47.77%), SOD and POD were able to keep MDA at the level of CK (D0). Yet it was difficult for them to prevent obvious injury from lipid peroxidation if the drought time prolonged. On the other hand, the proportion of various endocyte (Pro, soluble protein, soluble sugar) increased in the cytosol (especially during 9 ~ 18 d when the dramatic decline of soil water content happened) to maintain the cell turgor, to strengthen water absorption and transport capacity (Tyree & Hammel, 1972). They were even believed to help remove ROS and stabilize membrane and protein structure (Smirnoff, 1998;Rathinasabapathi, 2000; Shvaleva et al, 2005). While for *A. cremastogyne*, the leaves remained on the plant could get ampler water in the process of drought for 18 d because of its lower transpiration rate and defoliation strategy, therefore the osmoregulation and antioxidative metabolism were not so strong as those of *E. grandis*, the change of MDA content was not significant, implying that the oxidation threat was not serious to *A. cremastogyne*. However, the reactions of osmolytes and antioxidants were more sensitive (e.g. H_2O_2, SOD, Pro and soluble protein of *A. cremastogyne* were already significant changed in D3). When the stress time reached 15 ~ 18 d, SOD, soluble sugar and soluble protein in *A. cremastogyne* leaves rebounded more or less, demonstrating that obvious threat of dehydration and peroxidation might also happen to it if the treatment time exceeded 18 d .

4.3 Productivity of E. grandis and A. cremastogyne under the Continuous Drought Condition

Increase of WUE of plants was often found under drought stress, but it was considered to be at the cost of slower growth, as Heitholt (1989) believed. Ma et al (2002) found that *Eucalyptus* species consumed more water to keep their higher assimilation capacity in order to adapt to hot-dry environment, while the assimilation of several species of *Acacia* was lower together with the lesser consumption of water. *E. grandis* was likely to lay particular emphasis on photosynthetic production rather than water conservation in the balance of the two, thus it did not take measures of defoliation (Cowan, 1978).

Although the WUE (and the Pn in the later stage of drought stress) of a single leaf of *A. cremastogyne* was higher, it was evident that much smaller total leaf area might result in lower WUE at yield level compared with *E. grandis* (Wang & Liu, 2000). This was the reason why *A. cremastogyne* suffered greater inhibition in growth. When drought for 6 d, Gs and Tr of *A. cremastogyne* significantly increased probably on account of the optimal soil water content (17.72%) for the activity of the rhizobia, while too wet or too dry soil were not conducive to their growth and breeding (Wu, 1992).

5. Conclusion

In summary, under drought condition, *E. grandis* mainly took the measure of tolerance (increasing the contents of osmolytes and the activities of antioxidative enzymes) to ensure the absorption and the transport of water and prevent further damage caused by dehydration. It also took the strategy of drought avoidance by maintaining a high water holding capacity and reducing leaf stomatal conductance to reduce water loss. *A. cremastogyne* mainly adopted the drought avoidance strategy (defoliation, decrease of stomatal conductance) to reduce water consumption, whereas efforts on drought tolerance were hardly made. *E. grandis* retained most of its leaves to maintain the accumulation of photosynthetic products in face of water deficit, as a result, water consumption was relatively higher. So *E. grandis* might suffer great injury more easily with prolonged drought time. Thus in forest management, to ensure that 1 a seedlings of both species grow well, persistent drought of no more than 9 d was recommended to avoid too low soil moisture. Additionally, in the areas where annual rainfall is low and the seasonal distribution of which is uneven, such as the central and east regions of Sichuan Basin, one should be cautious to develop *E. grandis*, because the continuous drought often persists tens of days.

The death points of drought on these two species were not discovered in this study, their restorability after rewatering were not observed either. And whether or not the drought resistance would enhance if the age of plant increases? All these above remain to be more comprehensive in-depth studied so as to evaluate and compare their drought adaptabilities scientifically.

Acknowledgements

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Table 1. The experiment design

Drought treatment	(time)						
	10.09	10.12	10.15	10.18	10.21	10.24	10.27
D0	+	+	+	+	+	+	+
D3	+	+	+	+	+	+	−
D6	+	+	+	+	+	−	−
D9	+	+	+	+	−	−	−
D12	+	+	+	−	−	−	−
D15	+	+	−	−	−	−	−
D18	+	−	−	−	−	−	−

Note: "+" denotes that watering was implemented on the day, "-" denotes that watering was not implemented.

Table 2. Height and surface diameter growth of *E. grandis* and *A. cremastogyne* seedlings under the condition of continuous drought stress

Water treatment	*E. grandis*		*A. cremastogyne*	
	Growth of height(cm)	Growth of surface diameter(mm)	Growth of height(cm)	Growth of surface diameter(mm)
D0	7.67±1.34 Aa	6.28±2.30 Aa	2.10±0.14 ABCb	4.25±1.37 Aa
D3	7.10±2.40 Aab	3.74±1.23 Aa	2.40±0.28 ABab	3.67±1.73 Aab
D6	5.77±1.82 ABab	6.84±0.24 Aa	3.70±1.98 Aa	2.06±0.67 Aab
D9	3.90±0.85 ABCbcd	4.90±2.14 Aa	2.37±2.37 ABab	3.23±1.78 Aab
D12	4.70±1.98 ABCabc	2.71±1.32 ABa	1.00±0.99 ABb	1.53±0.55 ABb
D15	2.03±1.53 BCcd	1.23±0.18 Cb	−3.90±0.42 Cc	2.31±0.27 Aab
D18	0.70±0.57 Cd	−0.98±1.17 BCb	−2.80±0.42 BCc	−1.37±1.15 Bc
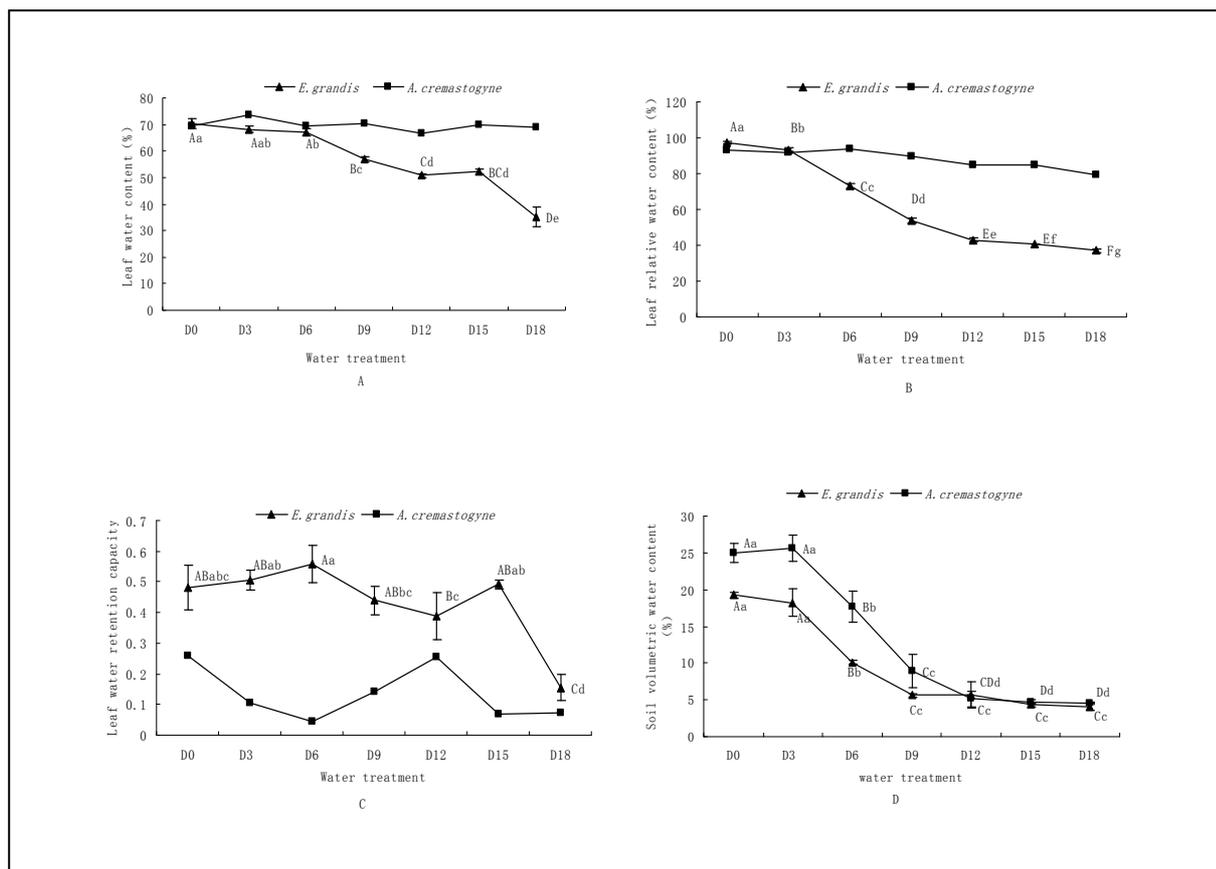
Figure 1. The change of soil water content and leaf water indices of *E. grandis* and *A. cremastogyne* under the condition of continuous drought stress

Note: the same line meant the significance was at the level of 0.01 and 0.05 respectively within mean values. The same as below.
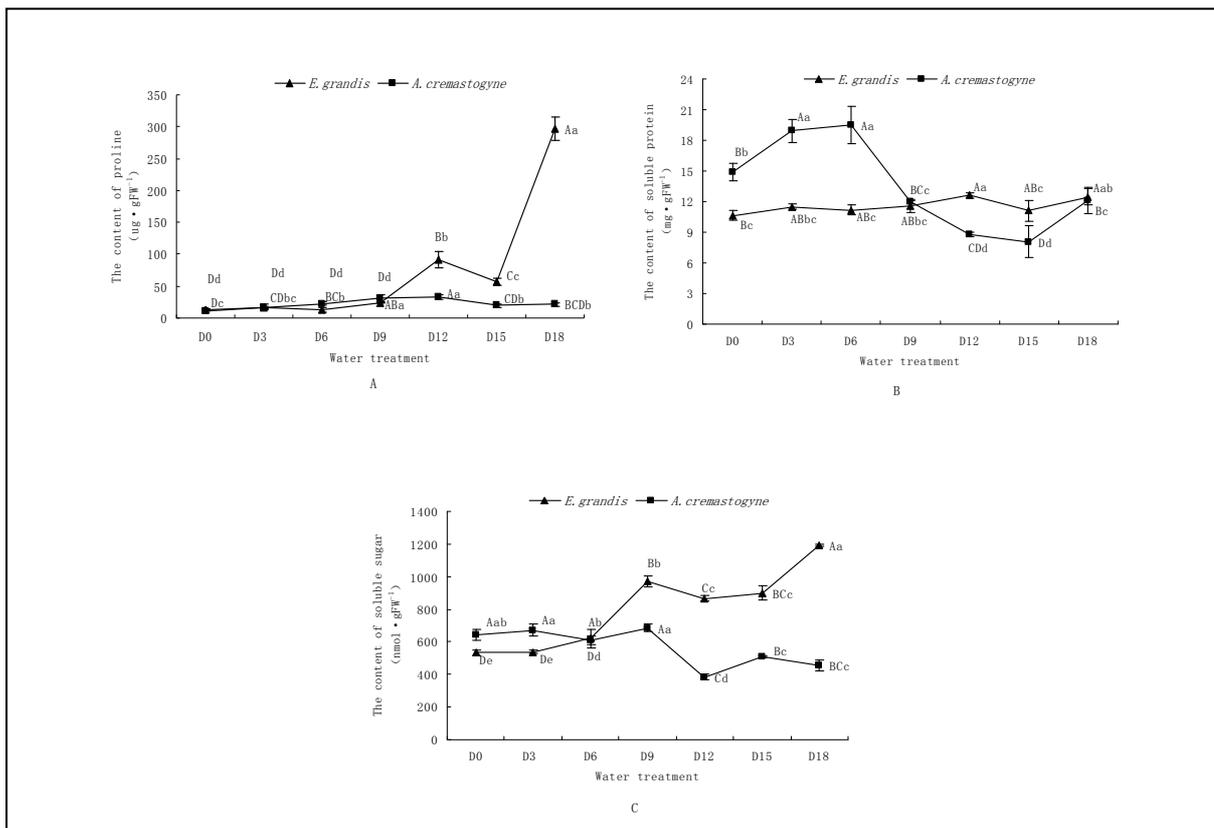
Figure 2. The response of three osmolytes of *E. grandis* and *A. cremastogyne* to continuous drought stress
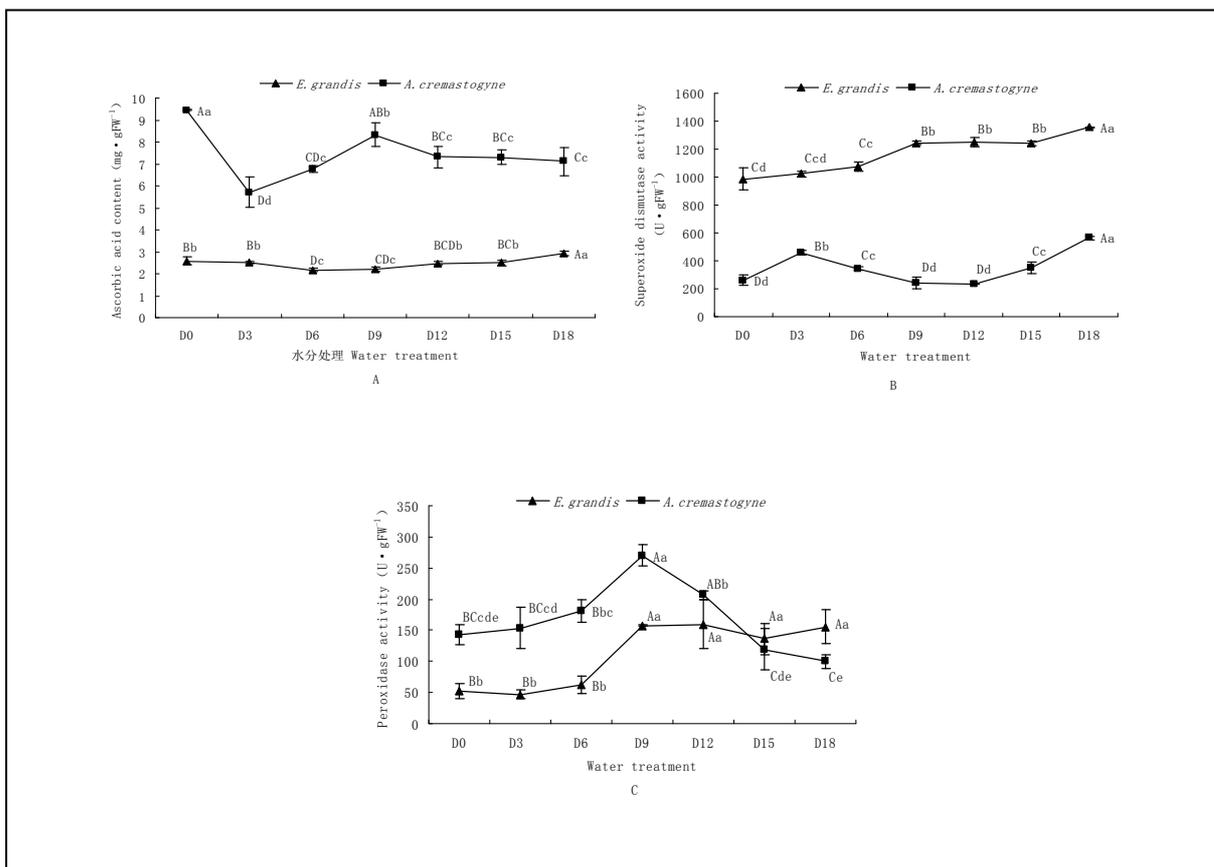
Figure 3. The effect of continuous drought stress on antioxidant system of *E. grandis* and *A. cremastogyne*
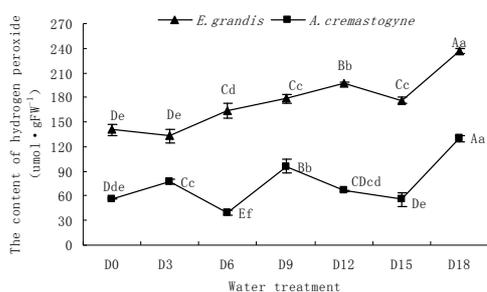
Figure 4. The response of H_2O_2 of *E. grandis* and *A. cremastogyne* leaves to continuous drought stress
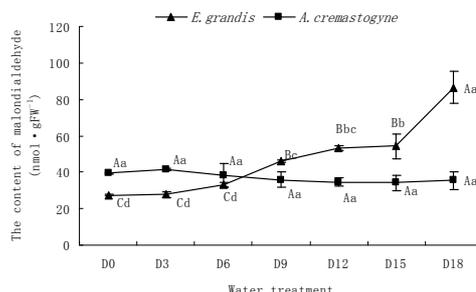
Figure 5. The response of MDA of *E. grandis* and *A. cremastogyne* leaves to continuous drought stress
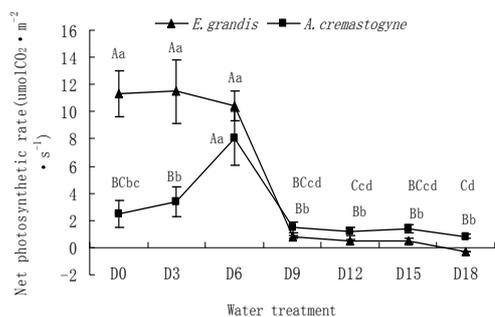
Figure 6. The response of net photosynthetic rate of *E. grandis* and *A. cremastogyne* to continuous drought stress
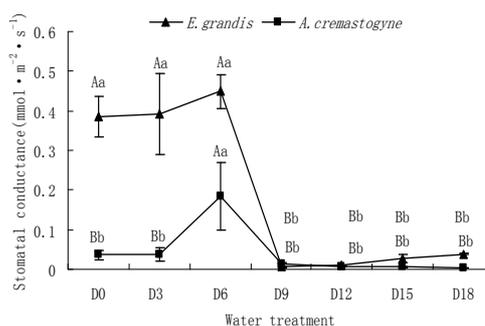
Figure 7. The response of Stomatal conductance of *E. grandis* and *A. cremastogyne* to continuous drought stress
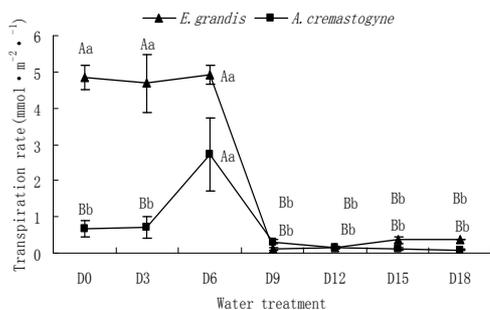
Figure 8. The response of transpiration rate of *E. grandis* and *A. cremastogyne* to continuous drought stress
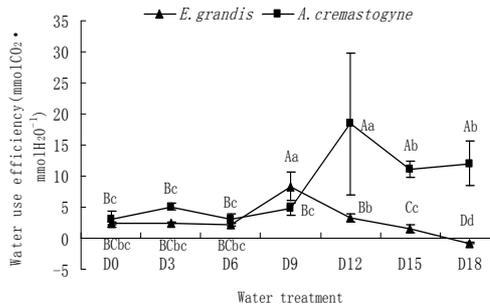
Figure 9. The response of water use efficiency of *E. grandis* and *A. cremastogyne* to continuous drought stress