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Biochemical and Physiological Responses of *Pterocarya stenoptera* and *Taxodium ascendens* Saplings to Different Configuration Modes and Water Regimes*

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Abstract: Three different kinds of water treatment and two configuration modes were applied to examine the biochemical and physiological responses of *P. stenoptera* and *T. ascendens* saplings under simulated vegetation of the Hydro-fluctuation Belt of the Three Gorges Reservoir Region. The water treatments were normal growth water condition (CK), light drought water stress (LD) and water submersion (SM). The first configuration mode was 3 *P. stenoptera* and 6 *T. ascendens*, and the second was a reverse of the number of saplings of both species. The results showed that different configuration modes could not significantly influence the photosynthetic pigment, SOD, POD, CAT, ASP, $O_2 \cdot^-$, proline and soluble protein of the two species. In contrast, different water treatment and time had significant influences on SOD, POD, CAT, ASP, $O_2 \cdot^-$ and soluble protein of the two species. The interaction of configuration modes × water treatment, configuration modes × time, configuration modes × water treatment × time had no significant influence on photosynthetic pigment, SOD, POD, CAT, ASP, $O_2 \cdot^-$ and soluble protein of the two species. However, the interaction of water treatment × time had significant influences on SOD, POD, ASP and $O_2 \cdot^-$ of *P. stenoptera*, as well as on SOD, CAT, ASP and proline content of *T. ascendens*. Our study showed that both species had to some extent different biochemical and physiological responses to water stress, and were relatively well-adapted to the dynamic hydrological environment in the hydro-fluctuation belt of the Three Gorges Reservoir Region.

Key words: Three Gorges Reservoir Region; configuration mode; water tress; physiological response; *Pterocarya stenoptera*; *Taxodium ascendens*

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Given a water level rise of 145 ~ 175 m, a large area of hydro-fluctuation zone has been formed by the impoundment of the Three Gorges Reservoir (TGR). As an active belt of exchange of energy, materials and information between terrestrial ecosystems and the water ecosystem, this area is vulnerable to external changes[1], having a larger flooded area, longer flood duration, and deeper flood depth. Furthermore, the flood season has been altered to winter from summer. These changes have caused a considerable area of degraded

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vegetation in the TGR, threatening its landscape quality, soil and water conservation and environmental health [2,3]. At present, engineering and biological measures are the main methods to restore damaged riparian zones. Compared with the huge cost of engineering measures, bioremediation is more suitable and has fewer negative impacts [4]. Therefore, to construct vegetation in the fluctuation zone has become an important way to restore the degraded ecosystems of the TGR.

Since the impoundment of the TGR, periodic flooding in the fluctuation zone has caused hypoxia in coastal plants, hindering vegetation construction and conservation in the zone. Changes of soil water content in response to periodic flooding must have impacts on the growth and ecophysiological characteristics of the afforestation tree species *Taxodium ascendens* and the native species *Pterocarya stenoptera*. Domestic and international research has focused on the respective biological characteristics [5-6], growth and yield [7-8], planting density [9-11], wood physical properties [12-14] and ecophysiological features [15-22] of these two species. Earlier studies have only addressed the influence of flooding on single species, but we cannot use a single species to restore vegetation. Thus, a co-existence of species, namely multi-species configurations should be utilized, not only for efficient utilization of space, but also as a way to evade pests and diseases. However, many factors such as species competitiveness, community density and species proportion affect competition among species [23]. Species with similar competitiveness could co-exist [24], but interspecific competition intensity becomes higher with increasing species density [25]. Hence, coexistence could be promoted by reducing the proportion of species with high competitiveness [26]. Although the two adaptive species studied here could co-exist whether or not under simulated soil water changes in the hydro-fluctuation belt of the TGR, further study is still needed. Their physiological and biochemical responses under different configuration modes could provide direct reference for vegetation restoration in the TGR, and few studies about this have been documented.

1 Materials and methods

1.1 The research material

To provide consistent experimental conditions in this study, we chose *T. ascendens* and *P. stenoptera* of the same growth condition with a height of 1.2 m. Sixty three specimens of *P. stenoptera* and *T. ascendens* respectively, 288 *Salix variegate* and 324 *Cynodon dactylon* (Linn.) Pers and *Acorus calamus* Linn, respectively, were planted in 18 pools of 2 m×2 m×1 m on January 15 ~ 18, 2011, at Geleshan Academy of Forestry in Chongqing. The planting pattern is shown in Fig. 1. 50 cm depth of soil was initially set at the bottom of the pool. Nine seedlings at 0.7 m×0.7 m plant spacing were planted in each pool, evenly distributed among shrubs and grasses. In order to make the experimental condition similar with that of hydro-fluctuation belt of the TGR, no daily field management was carried out, and water treatment started from May after natural restoration of the plants.

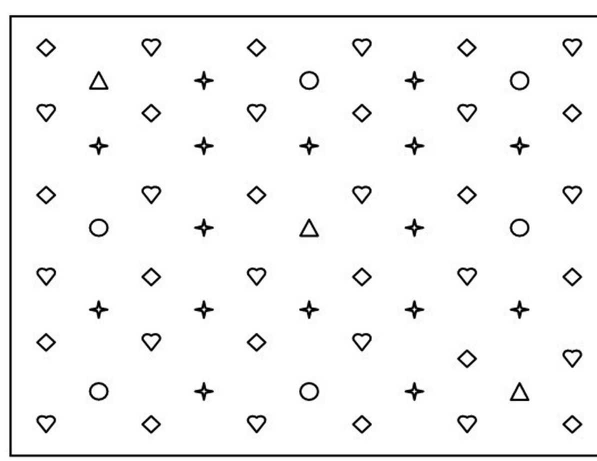

Fig. 1 Sketch map of the planting method of trees, shrub and herbs

○ represents dominant trees, △ represents auxiliary trees, ♡ represents *Salix variegata* Franch, ◇ represents *C. dactylon*, ✦ represents *A. calamus*

1.2 Experiment design

Two configuration modes, combining dominant tree species and auxiliary species in each mode, were implemented in this study (Tab. 1). There were three water treatments with three replications: normal water supply (CK), mild drought group (LD) and flood above 30 cm of soil surface (SM) [27-28].

Tab. 1 Two different configuration modes

Configuration mode	Tree	shrub	herbaceous
1	3 *P. stenoptera* +6 *T. ascendens*	18 *S. variegata*	8 *C. dactylon*+8 *A. calamus*
2	6 *P. stenoptera* +3 *T. ascendens*	18 *S. variegata*	8 *C. dactylon*+8 *A. calamus*

A processing period of ten days was used after May when the treatment started. Physiological and biochemical indices were determined continuously. There were five time-determinations with three replications respectively in total.

1.3 Determination of leaf physiological indices

Fresh leaves of the plants were selected, cut, and put into ziplock bags after removing the air, followed by icing in pre-prepared ice boxes and storing at -80℃ for further testing.

Chlorophyll content was determined by extraction [29]. Superoxide dismutase (SOD) activity, peroxidase (POD) activity, superoxide anion ($O_2 \cdot^-$) and soluble protein were measured using the method of Jun-feng Gao [30]. Catalase (CAT) and ascorbate peroxidase (ASP) activity were determined using the method of Yi-shun Zhang [31] and literature [32] respectively. Free proline content was measured by sulfosalicylic acid [30].

1.4 Data analysis

Multi-factor analysis of variance was used to reveal the effect of configuration, moisture, time and their interactions on the physiological and biochemical characteristics (GLM, SPSS16.0) of *P. stenoptera* and *T. ascendens*. Duncan's multiple comparisons ($\alpha = 0.05$) were utilized to test significant differences between each index. Figures were made using Microsoft Office Excel 2003.

2 Results

2.1 The change of chlorophyll content

Results of ANOVA (Tab. 2, Tab. 3) showed different impacts of configuration mode, water treatment, time, and their interactions on the physiological and biochemical responses of the plants. Chl a and Chl b content of *P. stenoptera* in LD was the highest, and that in SM was significantly lower than the control over time (Fig. 2, Fig. 3). There was a similar change of Chl a in terms of *T. ascendens* but no significant effect was found in soil moisture on Chl b content (Fig. 2, Fig. 3).

Tab. 2 Results of analysis of variance (ANOVA) testing for main effects of configuration mode, water treatment, time, and their interactions on physiological and biochemical responses of *P. stenoptera*

Projects	Physiological and biochemical indicators of *P. stenoptera*								
	Chl a	Chl b	SOD	POD	CAT	ASP	$O_2 \cdot^-$	proline	soluble protein
Configuration	0.714	0.711	0.986	0.298	0.934	0.833	0.606	0.981	0.121
Treatment	0.011	0.000	0.000	0.000	0.000	0.000	0.000	0.133	0.000
Time	0.391	0.029	0.000	0.000	0.000	0.000	0.000	0.007	0.000
Configuration×Treatment	0.974	0.926	0.883	0.995	0.851	0.918	0.879	0.914	0.978
Treatment×Time	0.906	0.623	0.001	0.000	0.939	0.005	0.044	0.501	0.001
Configuration×Time	1.000	0.995	0.517	0.001	0.988	0.992	0.989	0.958	0.891
Configuration×Treatment×Time	1.000	1.000	0.999	0.206	0.999	0.990	0.999	1.000	0.998

Note: The data in the Table are p value, the same below.

Tab. 3. Results of analysis of variance (ANOVA) testing for main effects of configuration mode, water treatment, time, and their interactions on physiological and biochemical responses of *T. ascendens*

Projects	physiological and biochemical indicators of *T. ascendens*								
	Chl a	Chl b	SOD	POD	CAT	ASP	$O_2\cdot^-$	proline	soluble protein
Configuration	0.940	0.444	0.607	0.539	0.954	0.637	0.755	0.962	0.301
Treatment	0.007	0.626	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Time	0.098	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000
Configuration×Treatment	0.976	0.991	0.422	0.960	0.977	0.762	0.990	0.095	0.940
Treatment×Time	0.957	0.239	0.000	0.899	0.000	0.003	0.676	0.000	0.002
Configuration×Time	1.000	1.000	0.977	0.934	0.733	0.998	0.996	0.051	0.997
Configuration×Treatment×Time	1.000	1.000	0.669	0.996	0.895	0.998	1.000	0.025	0.995

2.2 The change of superoxide anion content

Different impacts of configuration mode, water treatment, time, and their interactions on the two species are shown in Tab. 2 and Tab. 3. Both mild drought and flooding significantly increased superoxide anion content of *P. stenoptera* and *T. ascendens* (Fig. 4).

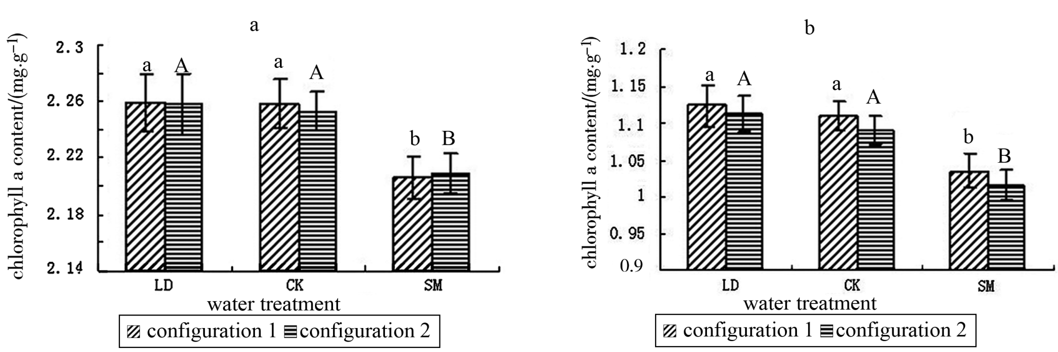

Fig. 2 Effects of water treatment on chlorophyll a of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

The letters on each column indicate the significance of different water treatment under the same configuration mode ($\alpha = 0.05$). The lower-case letters are for configuration mode1 while upper-case letters are for configuration mode2. The same below.

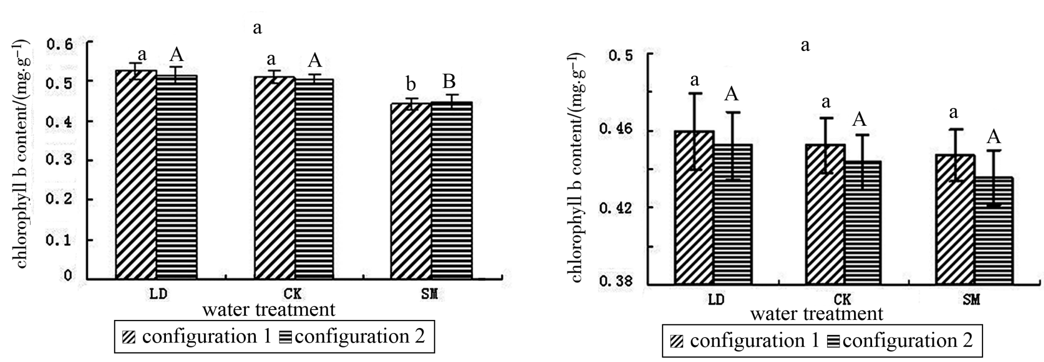

Fig. 3 Effects of water treatment on chlorophyll b of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

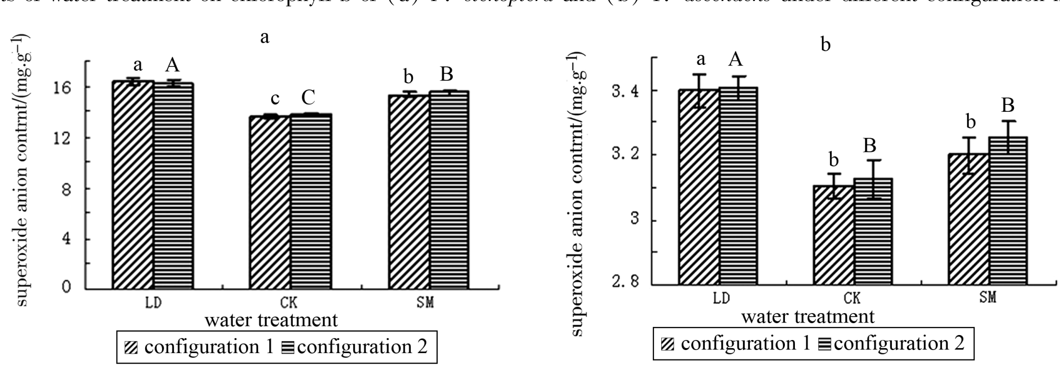

Fig. 4 Effects of water treatment on superoxide anion of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

2.3 The change of protective enzyme activities

Configuration had no significant impact on SOD, POD, CAT and ASP activity of *P. stenoptera* and *T. ascendens*, while water treatment and time had significant impacts on them respectively (Tab. 2, Tab. 3). The interaction of configuration and water treatment had no significant influence on them, while the interaction of water treatment and time had significant impacts on SOD, POD and ASP activity of *P. stenoptera* and SOD, CAT and ASP of *T. ascendens*. Interestingly, interaction of configuration and time only had extremely significant impact on the POD activity of *P. stenoptera*, while no significant impact was found for other enzymes of the two species. In addition, activities of SOD and POD in LD and SM of *P. astenoptera* and *T. ascendens* were significantly higher than their control group (Fig. 5, Fig. 6). On the contrary, the activities of CAT in LD and SM of both species were significantly lower than controls (Fig. 7). Compared with CK, the activity of ASP in LD of both species significantly increased but no comparable difference was found for *P. stenoptera* in SM, which is in contrast with the significant increase in ASP for *T. ascendens* in SM (Fig. 8).

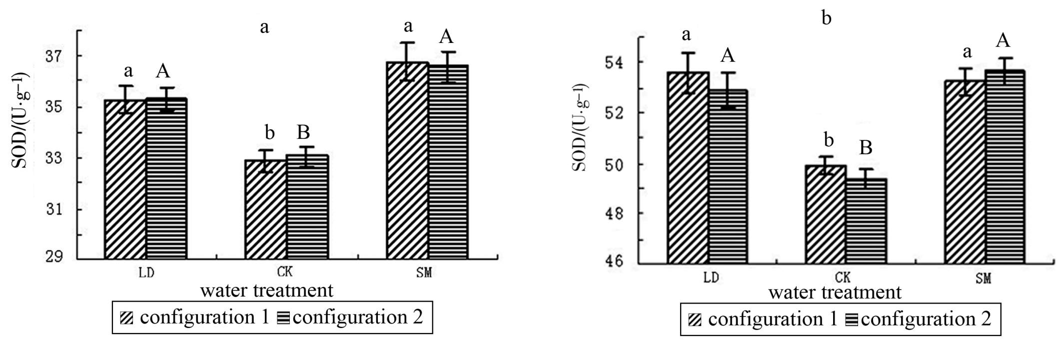

Fig. 5 Effects of water treatment on superoxide dismutase of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

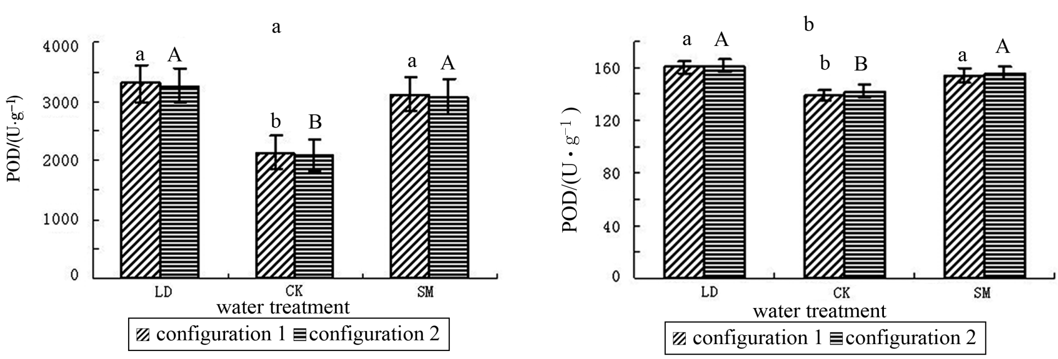

Fig. 6 Effects of water treatment on peroxidase of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

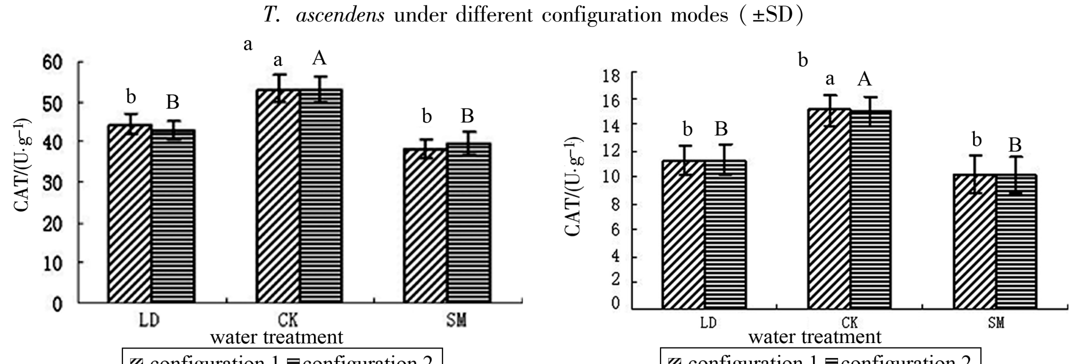

Fig. 7 Effects of water treatment on catalase of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

2.4 The change of osmotic adjustment substance content

Proline contents of *P. stenoptera* and *T. ascendens* were not significantly affected by configuration, while water treatment, time and their interactions had significant impacts on that of *T. ascendens* (Tab. 2, Tab. 3). Analysis of variance also showed that both mild drought and flooding increased free proline content significantly, and the content in *P. stenoptera* in LD was similar to SM, making a sharp contrast to a significant difference between both groups in the case of *T. ascendens* (Fig. 9).

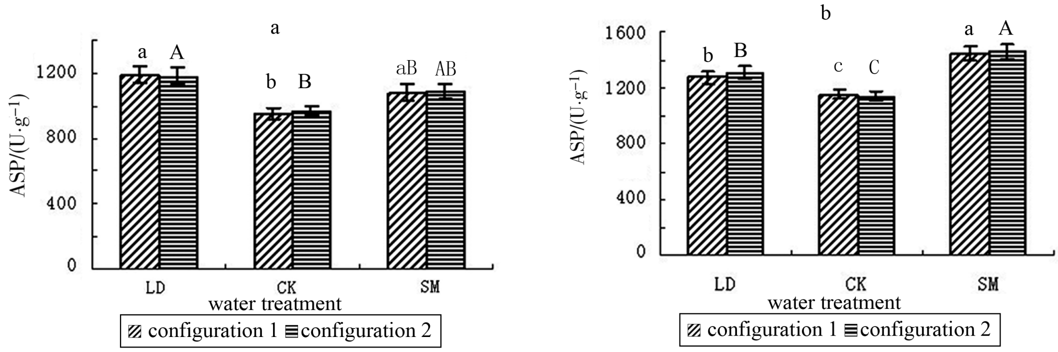

Fig. 8 Effects of water treatment on ascorbate peroxidase of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

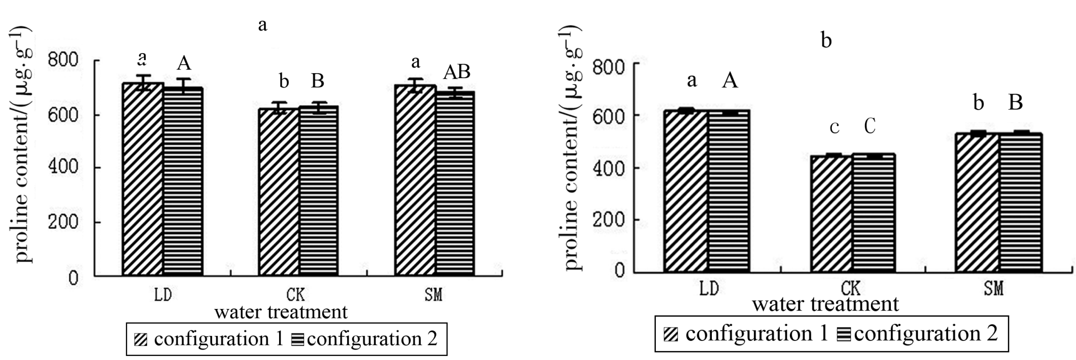

Fig. 9 Effects of water treatment on proline of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

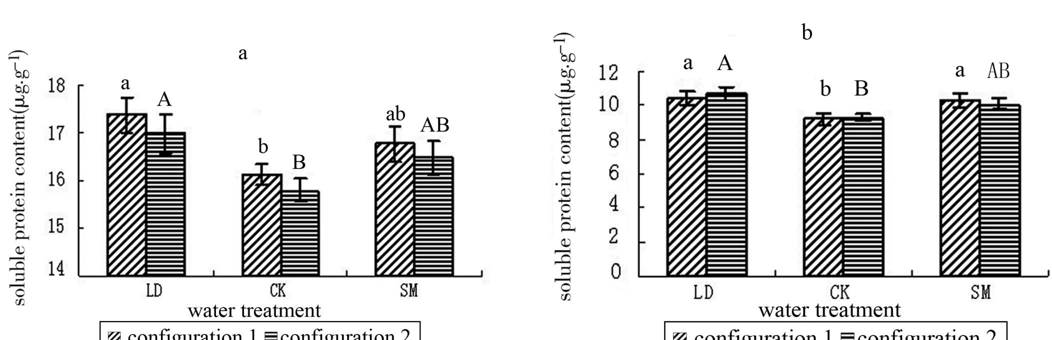

Fig. 10 Effects of water treatment on soluble protein of (a) *P. stenoptera* and (b) *T. ascendens* under different configuration modes (±SD)

Likewise, configuration had no significant impact on soluble protein content, with contrast to a significant impact of water treatment, time and their interactions on proline of *T. ascendens* (Tab. 2, Tab. 3). The content of soluble protein in LD of both species significantly increased compared with their control groups but no difference existed between SM and CK (Fig. 10).

3 Discussion

Sustainable flooding and short-time drought in the

TGR are likely to affect plant photosynthesis. Photosynthetic pigments, playing an essential role in photosynthesis, are mainly involved in light energy absorption, transmission and conversion, and plants alter their pigment ratio in adapting to their surroundings [33, 34]. Previous research has shown that plants enhance the content of photosynthetic pigments to withstand the adverse environment in a short-term mild drought [35-36]. While in a condition of flooding when plants confront hypoxia, free radicals are accumulated, affecting biosynthetic pathways. Chlorophyll content is then decreased by accelerated chlorophyll degradation caused by reactive oxygen [36-37, 19]. Our research found that Chl a and Chl b of *P. stenoptera* in mild drought were increased but not significantly, while in flooding they were significantly decreased. Mild drought did not significantly increase chlorophyll contents of *T. ascendens*. However, its Chl a content was significantly reduced under flooding, while no effects of flooding was found for Chl b. This may be due to a more sensitive response to stress in terms of Chl a than Chl b. Drought and flooding stresses have resulted in a change of chlorophyll contents, and photosynthetic pigment contents could also be influenced by planting density [38] and affect plant growth ultimately. On the other hand, water stress will lead to plant growth rate decline, leaf senescence and loss, chlorophyll degradation [35, 37] or even digestion [39], descent of photosynthetic rate [40] and plasmalemma damage [41], and contribute to plant death under inordinate stress. The mechanisms of plants to fight against adversity lie in two aspects, enhancing activity of protective enzymes such as SOD, POD, CAT to remove excessive reactive oxygen [42], and increasing osmotic adjustment substances like proline to maintain the stability of osmotic potential. Smirnoff confirmed that applying proline can remove excessive reactive oxygen [43]. Proline accumulation has been reported to be an adaptive plant response [44].

Lin considered that the different responses to stress of plants derived from the differences of their antioxidant capacity [45]. Our results suggest that the content of superoxide anion of *P. stenoptera* was significantly increased in mild drought and flooding, which is similar to the results of research on red pine by Xiufeng Yan [46] and white birch by Guo-rong Sun [47]. That of *T. ascendens* was significantly increased in mild drought, but no significant change under flooding treatment, showing a good resistance to flooding of *T. ascendens*. Therefore, *T. ascendens* is more sensitive to drought than to flooding. Meanwhile, the change of SOD, POD, CAT, ASP activity of *P. stenoptera* and *T. ascendens* in LD and SM is similar those found by to La-mei Xia [48]. As an inducible enzyme, SOD activity is induced by concentration of substrate. In our study, drought and flooding promoted the release of superoxide anion, resulting in a series of variation.

In brief, both *P. stenoptera* and *T. ascendens* respond to water stress positively, both physiologically and biochemically. They can adapt to mild drought and flooding. *P. stenoptera* displayed a better tolerance to drought than to flooding, while *T. ascendens* is just the opposite. Therefore, *P. stenoptera* could be planted at places higher in the fluctuation zone, while *T. ascendens* would be planted lower in the zone for the purpose of vegetation restoration. In our study, the two configuration modes affected no indices of *P. stenoptera* and *T. ascendens* significantly. This may be caused by a limitation of time such that no competitive inhibition had been formed. This issue will require future research.

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