Physiological Acclimation of Taxodium Hybrid ‘Zhongshanshan 118’ Plants to Short-term Drought Stress and Recovery

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Abstract. The physiological acclimation of Taxodium hybrid ‘zhongshanshan 118’ (T.118) plants to a progressive drought stress and drought-stressed to recovery treatment (DS-R) was investigated in this study. Plants of control (C) treatment were watered daily throughout the experiment. Results indicated that water deficit reduced stomatal conductance (gs) to improve water use efficiency (WUE) and, as a consequence, net photosynthetic rate (Pn), transpiration rate (Tr), and intercellular CO2 concentration (Ci) were also decreased in DS-R T.118 plants compared with C plants. These reductions became more significant with decreasing soil water availability. Correlation analysis showed gs was positively correlated (P < 0.01) with the soil water content as well as leaf relative water content (RWC). There was a tendency to accumulate proline, malondialdehyde (MDA), antioxidases, and membrane electrolyte leakage as stress intensity increased. Moreover, drought stress induced significant (P < 0.05) decline in total chlorophyll contents (Chl t) and increase of nonphotochemical quenching (NPQ) on day 8 as a protective mechanism. Cluster analysis distinguished the adaption of T.118 plants to water deficit in two ways. First, photosynthesis was related to thermal dissipation, and second antioxidation was related to morphology and osmosis. Furthermore, tested parameters showed a reversed tendency and restored equivalently to C levels after 9 days of rewatering. These findings suggest that T.118 plants demonstrated considerable tolerance to short-term drought stress and recovery due to a high degree of plasticity in physiological acclimation.

Drought is defined as soil and/or atmospheric water deficit, and is one of the main factors determining plants growth, vitality, and productivity (McDowell, 2011). According to scientific data, losses in growth and productivity of plants caused by water deficit may exceed the losses inflicted by all other adverse situations combined (Allen et al., 2010). More seriously, predictions of climate change for many regions reveal an intensification of water deficit, making it much more crucial to improve drought tolerance of plants.

Plants have evolved various mechanisms to confront low water availability (McDowell, 2011). Perennial herbs, for instance, can reduce water loss through inhibited transpiration by reducing leaf area, closing stomata, and leaf senescence (Gianoli and González-Teuber, 2005), which comes at a cost of reduced photosynthesis. Consequently, reduction of photosynthesis breaks the allocation balance of absorbed light, and thus conversion of photosystem II (PSII) is considered to dissipate light thermally by a radiationless process (Havaux and Tardy, 1999). Osmotic adjustment will occur under this circumstance to help maintain cell turgor and leaf water content within an operational range (Corcuera et al., 2002). The most studied osmotic compounds in plants are proline, glycine betaine, and sugars which act as a mediator and stabilizer (Verbruggen and Hermans, 2008). Meanwhile, abiotic stresses such as drought, salinity, chilling, and heavy metals will lead to oxidative stress (Selote and Khanna-Chopra, 2010).

Water deficit invariably leads to enhancement in reactive oxygen species (ROS), which will attack biological structures by damaging DNA, prompting the oxidation of amino acids and proteins, and provoking lipid peroxidation (Johnson et al., 2003). Currently, there is clear evidence that drought stress raises antioxidant defense pathways to protect cytomembrane from oxidative damage caused by dehydration, which exists in most of the subcellular compartments (Chaves et al., 2003; Liu et al., 2011).

As a matter of fact, tolerance of drought is defined as a comprehensive capacity integrating both drought and recovery (Chen et al., 2015). Despite the universal understanding of plant responses to drought, behaviors regarding rehydration are distinguished (Laanisto and Niinemets, 2015). Plant ability to resume photosynthesis, turgor pressure, leaf rehydration, and cellular metabolic function had been considered as postdrought recovery (Luo et al., 2011). Meanwhile, plant carbon balance and subsequent bio-physiological restoration depends on the rates and extent of photosynthesis recovery (Chen et al., 2015; Souza et al., 2004). For drought-tolerant species, this defense and adaptation to water deficit will be rapidly reversible when stress is relieved to avoid permanent dysfunction (Yazaki et al., 2015). Therefore, the recuperative potential from drought stress is a vital aspect in terms of plant vitality and survival (Gallé et al., 2007).

To improve drought tolerance, plant breeders often use hybridization to improve vigor and heterosis. These characteristics are commonly recognized as the superiority of hybrids relative to parental performance (Perron, 2008). To date, one of the most studied species is maize (Zea mays), which had been successfully exploited to enhance drought tolerance by creation of hybrids (Lopes et al., 2011). As demonstrated, horticulture plants in arid and semiarid regions are usually scarce, and drought-tolerant horticulture trees are crucial due to their unique services and resources in function and aesthetics, respectively (Fischer et al., 2009).

The genus Taxodium has historically been recognized as containing three species: Taxodium distichum (L.), Taxodium ascendens, Taxodium mucronatum (Tsumura et al., 1999). However, recent nomenclature places Taxodium as one species with three genotypes: T. distichum Rich. var. distichum (baldcypress-BC), T. distichum var. mexicanum (Carriere Gordon) (Montezuma cypress-MC) and T. distichum var. imbricarium (Nutt.) Croom (pondcypress-PC) (Adams et al., 2012; Creech et al., 2011; Denny and Arnold, 2007). For the purpose of this study, we have accepted the current nomenclature that combines all Taxodium associates into one species with three botanical varieties (Zhou et al., 2010). In China, ‘Zhongshanshan’ is an accepted term that describes the range of hybrids created by crosses between BC, MC, and PC. For instance, T.118 is the result of a backcrossed generation [(BC × MC) × MC] made in 1990s and selected in 2004 at the Nanjing Botanical Garden (Yin and Yu, 2005). Field afforestation of T.118 plants in this decade indicated heterobeltiosis in growth rate and environmental adaptability. Currently, T.118 plants have been widely used as timber, windbreak, and horticulture plantations in coastal and urban areas in southeastern China (Qi et al., 2014). As Creech et al. (2011) reported, Montezuma cypress can resist considerable magnitude drought stress. Meanwhile, a recent silviculture trial of T.118 plants in the hydro-fluctuation belt of the Three Gorges Reservoir, where soil conditions may be

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extremely dry after water levels drop, has demonstrated a survival rate greater than 90% (Yin et al., 2014). However, the ability of T. 7118 plants to cope with the anticipated decrease in water availability and its recuperative potential is still poorly understood.

Further understanding of physiology of both drought tolerance and recovery capacity will be instrumental in selecting specific biological traits for breeding. Thus, we assessed the temporal dynamics and interactions of T. 7118, which were based on successive decrease of soil moisture to identify changes induced by drought stress and recovery.

Material and Methods

Plant materials and experiment design. The experiment was carried out in a greenhouse located in Nanjing Botanical Garden (32°3’ N, 118°49’ E). Rooted cuttings of 2-year-old T. 7118 plants were grown under natural conditions from May to July 2015 in plastic pots containing 3:1:1 (v/v/v) clay, vermiculite, and perlite. Each plant was grown in a 7.2 L pot (23 cm upper diameter, 15 cm basal diameter, and 22 cm high) under fully irrigated conditions (27.2% v/v, ~75% of maximum pot capacity). They were allowed to acclimate to the natural conditions for 60 d before imposing the treatments. After the last watering on 26 July, 18 plants of similar growth (average 45 cm in height, 6.7 mm in basal diameter) were transferred to a greenhouse and assigned to two different treatments, each of which included nine replications. The C treatment was watered daily to previous pot capacity throughout the experiment. Drought-stressed to recovery (DS-R) treatment received no water irrigation for 8 d, after which all plants were rewatered daily to previous pot capacity for another 9 d for the recovery phase. The experiment was designed in a completely randomized block. The imposition of drought simulates what may be anticipated to occur in the field.

Temperature and relative humidity of the greenhouse was measured three times a day (0900, 1200, and 1600 HR). A sunshade net greenhouse was measured three times a day randomized block. The imposition of drought The experiment was designed in a completely

Fig. 1. Mean of daily temperature (°C) and relative humidity (%) in greenhouse.

Chlorophyll content. A total of 0.2 g fresh leaf was dispersed evenly in 95% ethyl alcohol at room temperature in the dark for 24 h. The Chl was calculated according to Ritchie (2008) by determining the absorbance at 649, 652, and 665 nm with a spectrophotometry (Jasco ultraviolet-visible, Japan). Specific leaf area (SLA) was calculated as the ratio of leaf area to leaf dry weight (DW). Leaves were weighed to determine the fresh weight (FW) and then floated on distilled water in petri dishes for 24 h to regain turgidity. After that, leaves were reweighed [turgid weight (TW)] without excess water and DW was measured after dried at 75 °C for 24 h (Roupheal et al., 2008). RWC was calculated as (FW – DW / TW – DW) × 100%.

Proline content. A total of 0.2 g fresh leaf was dispersed evenly in 3% aqueous sulfosalicylic acid and heated at 100 °C for 10 min. Concentration of proline was then determined in this extract by acid-ninhydrin reagent method on a DW basis (Bates et al., 1973).

Relative electrolyte leakage. A total of 0.2 g fresh leaf was immersed in a tube with 15 ml distilled water. The tube was centrifuged for 50 s and then electrical conductivity (EC1) of the extract was determined by a conductivity meter (DDS-11A, China). Thereafter, the tube was put in a boiling water bath for 10 min. After chilling to an ambient temperature, EC2 was measured. The relative electrolyte leakage (REL) of the leaf was calculated as EC1/EC2 (Blum and Ebercon, 1981).

Antioxidant enzyme activities and MDA. Under ice-cold conditions, 0.2 g fresh leaf was ground with 5 ml phosphate buffer (pH 7.8) and then centrifuged at the rotating speed of 12,000 rpm for 20 min at 4 °C. The supernatant was extracted to measure enzyme activities. Superoxide dismutase (SOD) activity was assayed by defining the amount of enzyme that inhibited the rate of nitroblue tetrazolium by 50% at 560 nm. Catalase (CAT) activity was assayed by measuring the induction of hydrogen peroxide at 1 min intervals in 3 min at 240 nm. The activity of peroxidase (POD) was determined with guaiacol as the substrate. An increase in absorbance was also recorded at 1 min intervals in 3 min at 470 nm. For measurement of MDA concentration, 2 ml of previous supernate was extracted with 0.67% thiobarbituric acid. The admixture was heated in a boiling water bath for 30 min and then promptly refrigerated in an ice bath before being centrifuged at 3000 rpm again for 10 min. The absorbance was recorded at 532, 600, and 450 nm, respectively (Sharma et al., 2011)

Photosynthesis and fluorescence. On days 5, 8, 10, and 17 of the experimental period, six of nine plants per treatment were chosen to measure net Pn, Tr, gs, and intercellular CO2 concentration (Ci). A red/blue LED light LI-6400 Portable System (LI-COR, Lincoln, NE) was used at a photosynthetically active radiation of 1200 μmol·m-2·s-1 quantum from 9:30 to 11:00 AM. CO2 concentration, air velocity, and humidity in leaf chamber were fixed at 380 μmol·mol-1, 0.5 L·min-1, and 60.0%, respectively. The measurement was performed on leaves chosen from branches that were center located. The ratio of Pn to Tr was calculated as instantaneous WUE.

After the test of photosynthesis, six of nine plants per treatment were chosen to measure chlorophyll fluorescence with a modulated fluorometer (PAM2100, Effeltrich, Germany). Leaves were irradiated by a saturated impulse (0.8 s) after a 30-min darkness adjustment during which the electron transport was considered to be ceased sufficiently in thylakoid membranes. The three parameters determined were the initial fluorescence (F0), maximum fluorescence (Fm), and the maximal photo-chemical efficiency of PSII, [Fv/Fm = (Fm – F0)/Fm]. Thereafter, leaves were exposed to a series of saturating pulses to measure the maximum fluorescence yield in the light-adapted period (Fm’). Actual PSII efficiency (Fv’/Fm) was then determined as (Fm’ – F0)/Fm’. Thereafter, leaves were exposed to a series of saturating pulses to measure the maximum fluorescence yield in the light-adapted period (Fm’). Actual PSII efficiency (φPSII) was then determined as (Fm’ – F0)/Fm’, where F0 was steady-state fluorescence. NPQ was derived from (Fm’ – F0)/Fm’ (Toscano et al., 2014).

Growth and biomass. At the end of the experiment on 11 Aug., six of nine T. 7118 plants were selected to measure the respective height and basal diameter by a tape line.
and vernier caliper, and then they were harvested and divided into leaves, stems, and roots, respectively. FW of leaves, stems, and roots was measured, and then they were dried at 75°C for 48 h until they reached a constant weight to measure the respective DW.

Statistical analysis. Differences of variables between treatments were tested using independent sample t test for means with unequal variances. Means were compared at a significance level ($P < 0.05$ and $P < 0.01$).

Results

Soil volumetric water content, greenhouse temperature and relative humidity. During the experiment, no significant fluctuations in daily temperature and relative humidity of greenhouse were observed (Fig. 1). On the first day after watered to pot capacity, soil water content was about 27.2% (v/v) in C and DS-R pots. Then, water content consecutively decreased in DS-R pots with drought duration (Fig. 2). On day 5, soil water content declined to 16.1% (v/v) and T.118 plants in DS-R treatment showed signs of wilting. On day 8, it declined to $\approx 11.1$% (v/v) and plants showed obvious wilting phenomenon; several leaves located on the top branches wilted irreversibly. Thereafter, all DS-R pots were watered to previous pot capacity, starting the recovery phase, while soil water content in C pots were maintained at about 27.2% (v/v) across the experiment.

Temporal dynamics of photosynthetic and fluorescent characteristics. Consistent with the daily temperature and relative humidity, T.118 plants in C treatment showed a stable trend of all measured photosynthetic parameters. Although in DS-R treatments, $P_n$, $g_S$, and $T_r$ of T.118 plants declined consistently with decreasing water availability during drought phase (Fig. 3A–C). $P_n$, $g_S$, and $T_r$ differed significantly ($P < 0.05$) between C and DS-R treatments on days 5 and 8. Especially on day 8, $P_n$, $g_S$, and $T_r$ in DS-R plants were reduced by 31.3%, 25.2%, and 23.1% compared with C treatment ($P < 0.01$), respectively.

![Fig. 2. Time course of pot volumetric water content in drought phase (mean and se, n = 9).](image1)

![Fig. 3. Variation in mean (and standard error, n = 6) (A) net photosynthetic rate ($P_n$), (B) stomatal conductance ($g_S$), (C) transpiration rate ($T_r$), and (D) intercellular CO$_2$ concentration ($C_i$) in T.118 of C (■) and DS-R (□) treatments over the course of the experiment. Each square represents samples measured on each sampling day. * *$P < 0.05$, $P < 0.01$ between treatments, respectively.](image2)
Data are mean ± SE (n = 3). Different lowercase letters in each column indicate significant differences (P < 0.05) between treatments on each sampling day.

Table 1. Fluorescence characteristics of T.118 plants during the experiment.

| Days | Treatments | Fv/Fm | qPSII | NPQ |
|------|------------|-------|-------|-----|
| 5    | C          | 0.83 ± 0.01 a | 0.78 ± 0.01 a | 1.56 ± 0.08 a |
|      | DS-R       | 0.81 ± 0.01 a | 0.73 ± 0.01 b | 1.67 ± 0.06 a |
| 8    | C          | 0.83 ± 0.01 a | 0.78 ± 0.01 a | 1.54 ± 0.04 b |
|      | DS-R       | 0.79 ± 0.01 b | 0.62 ± 0.01 b | 2.01 ± 0.02 a |
| Recovery 10 C | 0.83 ± 0.01 a | 0.78 ± 0.01 a | 1.55 ± 0.04 a |
|      | DS-R       | 0.81 ± 0.01 a | 0.78 ± 0.01 a | 1.66 ± 0.05 a |
| 17   | C          | 0.83 ± 0.01 a | 0.78 ± 0.01 a | 1.58 ± 0.02 a |
|      | DS-R       | 0.83 ± 0.01 a | 0.78 ± 0.01 a | 1.58 ± 0.02 a |

C = control; DS-R = drought-stressed to recovery; NPQ = nonphotochemical quenching; PSII = photosystem II.

Data are mean ± se (n = 6). Different lowercase letters in each column indicate significant differences (P < 0.05) between treatments on each sampling day.

Table 2. Water use efficiency (WUE), relative water content (RWC), specific leaf area (SLA), total chlorophyll contents (Chl), proline contents, and relative electrolyte leakage (REL) in T.118 plants.

| Days | Treatments | WUE (mmol·mol⁻¹) | RWC (%) | SLA (cm²·g⁻¹) | Chl (mg·g⁻¹ DW) | Proline (µg·g⁻¹ DW) | REL (%) |
|------|------------|------------------|---------|---------------|-----------------|---------------------|--------|
| 5    | C          | 2.7 ± 0.13 b     | 89.7 ± 0.7 a | 60.4 ± 1.23 a | 0.934 ± 0.09 a | 73.8 ± 4.6 b       | 24.4 ± 1.37 b |
|      | DS-R       | 3.2 ± 0.16 a     | 83.4 ± 1.2 a | 52.7 ± 0.73 b | 1.06 ± 0.06 a  | 106 ± 7.9 a        | 46.2 ± 1.71 a |
| 8    | C          | 2.7 ± 0.11 b     | 90.0 ± 2.2 a | 60.2 ± 1.77 a | 0.927 ± 0.18 a | 73.5 ± 4.4 b       | 24.5 ± 3.73 b |
|      | DS-R       | 3.2 ± 0.47 a     | 56.5 ± 2.3 b | 51.3 ± 0.52 b | 0.831 ± 0.06 b | 150 ± 7.3 a        | 52.4 ± 3.62 a |
| Recovery 10 C | 2.8 ± 0.08 b | 89.5 ± 0.75 a | 60.3 ± 1.19 a | 0.928 ± 0.03 a | 73.9 ± 4.0 b | 24.9 ± 3.62 a |
|      | DS-R       | 3.2 ± 0.25 a     | 81.5 ± 2.5 b | 55.9 ± 1.45 b | 0.917 ± 0.02 a | 117 ± 5.0 a        | 38.1 ± 5.87 a |
| 17   | C          | 2.7 ± 0.24 b     | 89.7 ± 3.3 a | 59.8 ± 1.93 a | 0.934 ± 0.06 a | 73.7 ± 3.5 a       | 25.5 ± 2.99 a |
|      | DS-R       | 2.7 ± 0.35 b     | 89.7 ± 1.2 a | 60.7 ± 1.24 a | 0.934 ± 0.06 a | 71.5 ± 2.7 a       | 18.9 ± 3.71 a |

C = control; DS-R = drought-stressed to recovery.

Discussion

The influence of water deficit on plant performance described in several species may be restrictive and even devastating (Álvarez et al., 2011; Liu et al., 2011). In our study, after 8 d of progressive drought stress, T.118 plants still survived. No significant difference in total day weight was observed between C and DS-R treatments. Besides, a significant increase of root to shoot ratio was found in DS-R plants compared with C plants after recovery. Such response is...
attributable to the development of root systems, because leaf and stem growth is relatively more inhibited than root. Generally under well-watered conditions, plants use a large fraction of light together with adequate moisture for photosynthesis. When drought is superimposed, the balance between light capture and water utilization is broken (Chaves et al., 2003). On one hand, T.118 plants enhanced WUE to cope with decreasing water availability. Thus, minimizing water loss in DS-R plants is essential and gs was reduced as initial acclimation for T.118 plants adapting to water deficit, which occurred together with a remarkable reduction in Tc. Furthermore, the dramatic reduction of Pn and gs and ongoing increase trend of Ci suggested the predominance of nonstomatal limitations to photosynthesis on day 8. Diminish of leaf RWC is another approach for plants to increase water exploitability, which may finally help to increase carbon investment to root tissues (Rosales-Serna et al., 2004). As expected, the leaves of DS-R T.118 plants exhibited large reductions in RWC, especially on day 8. During drought phase, several leaves located on the top branches of T.118 plants had been observed to wilt irreversibly due to over dehydration. In addition, correlation analysis suggested that T.118 plants lost part of leaf water content at low stomatal aperture.

On the other hand, superimposed drought stress makes it necessary for T.118 plants to subtract exscescent light, either preventing absorbing, for instance by rolling leaves to decrease leaf canopy or losing chlorophyll (Havaux and Tardy, 1999), or diverting internal light from photochemistry to thermal dissipation (Chaves et al., 2003). For DS-R plants, reduced SLA on days 5 and 8 was attributed to a strategy that allowed the minimization of light absorption. Meanwhile, decreasing of leaf Chl, accompanied by Fv/Fm and qPSII was observed despite the short duration of drought phase. Such photo-inhibition was probably associated with damage caused on the primary electron acceptors of PSII due to water deficit. What is more, photo-protection of the photosynthetic apparatus occurred in DS-R plants on day 8 since
To relieve drought-induced oxidative stress, stress broke such balance in mal growth conditions, the amount of ROS in the stress tolerance development. Under nondant enzymes activities, is indispensable for of plants, which mainly depends on antioxidant level induced by severe drought stress (Kishor et al., 2008). At the same time, the antioxidant level dramatically from days 5 to 8. So the accu-
dated proline minimized peroxidation to avoid membrane injury by maintaining cel-
ular turgor (Hare and Cress, 1997). In this experiment, membrane electrolyte leakage of T.118 plants had been detected as drought stress, since their photosynthesis was rap-
plied and DS-R (D) treatments. Each square represents samples measured on each sampling day. Bars indicate mean ± se, the replications of gs, soil water content, and RWC were 6, 6, and 3, respectively.

NPQ, which represents the light energy dissipated as heat, reached a maximum value and was significantly higher (P < 0.05) than C plants. This result was consistent with previous studies that a similar pattern of photo-protection was employed by barley (Hordeum vulgare) and mango (Mangifera indica), and such a mechanism was more active in drought-tolerant plants (Elsheery and Cao, 2008; Kocheva et al., 2004).

Among the compatible solutes in osmotic adjustment, proline plays a protective role to avoid membrane injury by maintaining cellular turgor (Hare and Cress, 1997). In this experiment, membrane electrolyte leakage of T.118 plants had been detected as drought stress, and contents of proline increased dramatically from days 5 to 8. So the accu-
mulated proline minimized peroxidation to preserve the protein structure and cell mem-
brane integrity in T.118 plants, which indicated remarkable alleviation of cellular hyperosmolarity and ionic disequilibrium inducted by severe drought stress (Kishor et al., 2005). At the same time, the antioxidant level of plants, which mainly depends on antioxid-
ent enzymes activities, is indispensable for the stress tolerance development. Under normal growth conditions, the amount of ROS in plants is low and steady. However, drought stress broke such balance in T.118 plants. To relieve drought-induced oxidative stress, SOD plays a key role in the first threshold of oxidative defense in catalyzing the dismutation of O2− into H2O2, and then the generated H2O2 will be eliminated by CAT and POD (Liu et al., 2011; Zhu et al., 2009). For DS-R T.118 plants, drought stress enhanced of SOD, POD, and CAT activities, exhibiting cooperative scavenging function to eliminate ROS. What is more, the antioxidant enzymes activities provided higher protection on day 8 against drought stress than day 5 since they kept increasing. Similar evidence of antioxid-
ids to water deficit had been observed in Brassica napus and Sinapis alba, registering low lipid peroxidation levels together with high antioxidant enzyme activities (Xia et al., 2016). In agreement with that, antioxidant enzyme activities in T.118 plants would play a vital role in achieving better water deficit tolerance.

Depending on the intensity of drought stress, the recovery of physiological charac-
teristics is progressive and will take several days or more at times (Souza et al., 2004). Although the recovery phase was limited to 9 d, during which T.118 plants did exhibit superior recuperative performance, 8 d of water deficit did not impair photosynthetic apparatus from the perspective of whole plants, since their photosynthesis was rap-
dly recovered after rewatering. As Sapeta et al. (2013) suggested, rapid and complete photosynthetic recovery from water deficit is a fundamental aspect of plant persistence, and dictates plant survivability in drought stress conditions with merely sporadic water supply. Likewise, completely restored photosynthesis were reported in other drought-
tolerant plants after a few days of rewatering, such as oleander (Nerium indicum) and psam-
moiphytes (Setaria viridis and Digtaria cil-
tataris) (Flexas et al., 2006; Luo et al., 2011). At the same time, Chl and fluorescence quickly recovered to pre-drought levels once stress had been relieved, indicating the previous damage caused on photochemistry had been successfully restored.

It is reported that recovery of plants from drought stress is a two-stage process (Xu et al., 2013). The primary stage occurs during the first few hours to reopen leaf stoma for gas and water exchange. The second stage lasts a few days, mainly normalizing metab-
olic function. In T.118 plants, following rewatering, the stomata reopened and the SLA and RWC recovered rapidly. The reason for the full recovery of leaf water relation in T.118 plants indicates that vessels were likely rapidly refilled by water. However, a drought tolerant strategy mainly based on reopened stomata was not efficacious enough to ensure rapid recovery (Zwicke et al., 2015). As cluster analysis demonstrated in this experiment, the collaborative defense of osmosis...
and antioxidation in T.118 plants played another emphasized part. The consistent decreasing trend of proline, SOD, POD, and CAT during recovery phase showed the successful restoration of osmosis and antioxidation. Moreover, persistence of T.118 plants can also be correlated with efficient regain of soil moisture by roots, since root to shoot ratio of T.118 in DS-R plants exhibited significant increase compared with C plants. Such advantage enhances soil acquisition and manifests a great plasticity in root system against drought stress (Padilla and Pugnaire, 2007).

**Conclusion**

Physiological acclimation of T.118 plants to short-term drought stress included elevated WUE by stomatal closure, decreasing of $P_{\text{m}}$, $T_{\text{c}}$, leaf area, Chl, and thermal dissipation. Meanwhile, accumulation of proline and antioxidases helped to maintain cell turgor and metabolic functions. After rewetting, these adaptions eventually returned to normal levels. Accordingly, we conclude that the T.118 plants have a high degree of plasticity in response to water deficit. These combined strategies enable T.118 plants to survive in short-term or stochastic drought conditions with unpredictable precipitation.

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