Lateral Root Traits of *Taxodium* Hybrid ‘Zhongshanshan 406’ in Response to Drought Stress

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Abstract. Roots are vital organs for resource uptake. However, the knowledge regarding the extent by which responses in roots influence plant resistance is still poorly known. In this study, we examined the morphological and physiological responses of lateral roots of *Taxodium* hybrid ‘Zhongshanshan 406’ (*Taxodium mucronatum*♀ and *Taxodium distichum*♂, T. 406) to 8 (DS-8) and 12 days (DS-12) drought. Control plants (CK-8 and CK-12) were well-watered throughout the experiment. Results indicated that drought resulted in significantly decreased root length, surface area, volume, and biomass and a relatively high death rate of roots (>2 mm). Specific root length (SRL) and specific root surface area (SRA) of drought-stressed T. 406 plants were reduced to enhance resource uptake. Meanwhile, root relative water content (RWC) of T. 406 plants in CK-12 treatment was 5.81 times of those in DS-12 treatment. Under drought stress and root superoxide dismutase and ascorbic acid (ASA) activities, proline and hydrogen peroxide (H₂O₂) contents consistently increased to benefit the elimination of O₂^{•−}. At the ultrastructural level, the organelle structure of T. 406 plant root tip was visibly damaged because of dehydration. The nucleus swelled and then exhibited uncommon features of disorganization and disruption. In short, our results provided substantial information about lateral root traits of T. 406 plants in response to drought stress, which is crucial to improve the drought resistance of *Taxodium* hybrid in the future breeding.

Over the large area of the Earth’s surface, drought is a major factor limiting plant productivity (Rowland et al., 2015). Tree roots play a key role in the drought tolerance but are relatively understudied compared with components of aboveground plant. Forest dieback as a result of increasing drought intensities has frequently been reported worldwide, from semiarid areas in Australia to Algeria in North Africa, and to Costa Rica in Latin America (Allen et al., 2010; Hartmann et al., 2015).

Given the impacts of drought, past causes and the possible mechanisms by which drought could occur in the future have received substantial attention. Studies have been implemented to explain tree death during drought from a physiological perspective (Allen et al., 2010; Diffenbaugh and Scherer, 2011). Plants can minimize water loss through inhibited transpiration by reducing leaf area and g_s , which comes at a cost of reduced photosynthesis. Currently, there are clear evidences that drought stress reduces

photosynthetic capacity and then inhibits plant productivity (Allen et al., 2010). Plant roots respond to environmental stresses by undergoing plastic morphological and physiological alteration (Doussan et al., 1999). Leaf transpiration drives water absorption through the root system, and root characteristics eventually determine plant access to water. Lateral roots are considered the main entrance point for water absorption, which might be greatly influenced by the traits of lateral root tips (Doussan et al., 1999). Ito et al. (2006) draw the conclusion that variations in the length of first-order lateral roots in responses to drought stress may help distribute their tips thoroughly in the soil. Thus, a potential xeromorphic response of plant root to reduced soil water availability is necessary to be examined.

The genus *Taxodium* is valuable for restoring degraded coastal areas, urban landscapes, and other greening projects (Creech et al., 2011). In China, *Taxodium* hybrid ‘Zhongshanshan 406’ (T. 406) is an accepted term that describes a hybrid selection created by a cross between *T. mucronatum* and *T. distichum*. The field afforestation of T. 406 plants from 2010 indicated heterobeltiosis in growth rate and environmental adaptation (Wu et al., 2016). T. 406 plants are insect-resistant and, therefore, considered promising in future forestry efforts in China. Although recent selection for drought resistance in *Taxodium* hybrids has primarily been based on physiological acclimation in leaf under

drought condition, the mechanisms in lateral roots by which *Taxodium* hybrids achieve potential high vitality under drought are not well understood.

For root measurements, Kano et al. (2011) used mainly root length as a significant parameter because of its determination of water and nutrient uptake. This study investigates several lateral root characteristics in T. 406 plants, aiming to detect adaptive responses of the root system to a decrease in water availability. Information about the ability of T. 406 plants to alter root traits under water stress could result in strategies of improved breeding for drought resistance in *Taxodium* hybrids.

Materials and Methods

The experiment was conducted in a greenhouse located in Nanjing Botanical Garden (32°3′N, 118°49′E). Rooted cuttings of 1-year-old T. 406 plants were grown in plastic pots of 7.2 L (23 cm upper diameter, 15 cm basal diameter, and 22 cm high) containing 3:1:1 (v/v/v) clay, vermiculite, and perlite under natural condition from June to Aug. 2016. They were allowed to acclimate for 70 d before imposing the drought treatment. After last watering on 23 Aug., 16 plants of similar growth (average 62.3 cm in height, 8.45 mm in basal diameter) were transferred to a greenhouse. A 50% sunshade net with uniform pore size was placed at the top of the greenhouse to avoid direct sunlight exposure. Control treatments were watered daily to previous pot capacity throughout the experiment. On days 8 and 12, four plants of each control treatment were separately taken from the respective pots and were set as CK-8 and CK-12 treatments, respectively. Drought-stressed plants did not receive irrigation and were sampled on 8 (DS-8) and 12 d (DS-12), respectively. Each treatment included four replications. A split-plot in a randomized complete block was used.

Temperature and relative humidity of the greenhouse were measured three times a day (0800 HR, 1200 HR, and 1600 HR). Soil water content was determined by oven-drying at 105 °C in DS-8 and DS-12 treatments after sampling (Hardie et al., 2014). During the experiment, mean daily temperature and relative humidity of greenhouse were 30.1 °C and 52.6%, respectively. On the first day after watered to pot capacity, soil water content was ≈26.2% in all pots. Then, the water content consecutively decreased in drought-stressed pots with drought duration. In DS-8 treatment, soil water content declined to 9.1% and T. 406 plants became wilt. In DS-12 treatment, water content reduced to 6.0% and the plants exhibited obvious wilting phenomenon with one-third of the leaves wilting irreversibly.

Biomass and root morphology analysis. Plant height and basal diameter were measured with a tapeline and a vernier caliper, respectively. Then, plants were separated into leaf, stem, and root and well washed. Lateral roots were spread apart on a water

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layer in a transparent tray (32 cm × 24 cm) for scan. The Epson scanning system was set at professional mode with reflective light at a resolution of 400 DPI. Thereafter, root images were analyzed for morphological traits of total length, surface area, volume, and their distribution in the root *n* diametric class, where *n* is 0–1, 1–2, and >2 mm, by WinRHIZO software (V5.0; Regent Instruments, Quebec, Canada) (Cavelier et al., 1992). After that, leaf, stem, and root were firstly dried at 105 °C for 30 min, then further dried at 75 °C for 48 h to obtain constant dry weight (DW). Specific root length and SRA were calculated as the ratio of total length/root DW and surface area/root DW, respectively. Root density was calculated as the ratio of root DW/volume. The root/shoot ratio was the result of underground parts/aboveground parts.

Root vigor, RWC, nitrogen, and phosphorus analysis. Root vigor was measured according to the triphenyltetrazolium chloride (TTC) method (Liu et al., 2008). Before TTC incubation, 1.00 g roots were washed thoroughly with distilled water for 10 min and placed in 10 mL of TTC reaction solution (0.5% TTC in 0.06 mol·L⁻¹ phosphoric buffer, pH 7.4) for 3 h at 37 °C in the dark. Then 2 mL of 1 mol·L⁻¹ H₂SO₄ was added to the tube to stop the reaction. The TTC solution was drained. Roots were ground and extracted with 10 mL of ethyl acetate and then the absorbance was read at 485 nm. For root RWC, selected fresh roots were weighed (FW) and then floated on distilled water in petri dishes for 24 h to regain turgidity (Rouphael et al., 2008). After that, roots were reweighed [turgid weight (TW)] without excess water and DW was measured. Root RWC was calculated as (FW – DW/TW – DW) × 100%.

Pulverized root samples of 0.10 g were digested in a digestion oven in concentrated H₂SO₄–H₂O₂ with mixed catalyst and then gradually heated to 400 °C until the contents became colorless. The extracting solution was analyzed with the Kjeldahl method (Kirk, 1950) and the Mo–Sb colorimetric method (Mel’Tser, 1960) to determine total nitrogen (TN) and total phosphorus (TP), respectively.

Proline, abscisic acid (ABA), and H₂O₂ determination. Fresh root tissue (0.20 g) was macerated in 3% aqueous sulfosalicylic acid and heated at 100 °C for 10 min for proline analysis. The contents of proline were then determined by the acid-ninhydrin reagent method on a FW basis (Bates et al., 1973). A sample of 0.20 g of root tip tissue was macerated in liquid nitrogen and 5 mL

of 80% (v/v) methanol was added. The extract was subsequently centrifuged at 4000 rpm at 4 °C. Abscisic acid was measured without further purification by an ELISA (ELISA; Jiancheng Institute of Biology and Engineering, Nanjing, China) using a monoclonal antibody. Absorbance values were recorded by a microplate reader (Bio-Tek; Winooski, VT) at 450 nm. The content of H₂O₂ was analyzed with an H₂O₂ assay kit (S0038; Beyotime). After adding the H₂O₂ lysate, test tubes with 50 µL sample solution were placed at room temperature for 30 min and then measured immediately with a microplate reader at a wavelength of 560 nm.

Antioxidase activities and ASA analysis. Each 0.2 g fresh root tip tissue was ground under ice-cold conditions with 5 mL phosphate buffer (pH 7.8) and then centrifuged at the rotation speed of 12,000 rpm for 20 min at 4 °C. Superoxide dismutase (SOD) activity was defined according to the amount of enzyme in the supernatant that inhibited the rate of nitroblue tetrazolium by 50% at 560 nm. To determine ASA concentration, each 0.50 g fresh root sample was homogenized in 5 mL oxalic acid-EDTA solution and centrifuged at 4000 rpm for 10 min at 4 °C. Then, 1 mL of supernatant was added to 5 mL oxalic acid-EDTA solution and 0.5 mL metaphosphoric-acetic acid, left for 10 min, and supplemented with 1 mL 5% H₂SO₄. Then, 2 mL 5% ammonium molybdate solution was added to react, then incubated at 30 °C for 15 min, and measured at an absorbance of 760 nm (Sharma et al., 2011).

Transmission electron microscopy (TEM). Preparation of the root tips required placement in glutaraldehyde fixative (2.5%) at pH 7.2 for 24 h under vacuum. After all roots were immersed in the fixative, each sample was rinsed three times in phosphoric acid buffer for at least 1 h. The samples were then treated according to Zhou et al. (2016) to observe and photograph root ultrastructure with a TEM (Hitachi, Japan) at 80 kV.

Statistical analysis. Means were compared by Duncan’s multiple-range test at the 5% level of probability by SPSS 19.0 (IBM Corporation, Somers, NY). Plotting was conducted with Origin 9.1 pro (Origin Laboratory, Northampton, MA).

Results

Root morphological traits. There were no significant differences in morphological traits between two control groups. Total length, surface area, and volume in DS-8 were 50.6%,

52.3%, and 52.8% lower (*P* < 0.05), respectively, than in DS-12 treatment. SRL and SRA exhibited a significantly decreasing pattern from DS-8 to DS-12 treatment (*P* < 0.05, Table 1). Total length, surface area, volume, SRL, and SRA in DS-12 treatment decreased to 27.3%, 29.0%, 20.5%, 17.7%, and 41.8% as compared with CK-12 treatment. The root density of DS-12 plants was significantly higher (*P* < 0.05) than those in CK and DS-8 plants.

Figure 1 showed the proportion of root traits in diametric classes. *T. 406* plants maintained a relatively high death rate of roots >2 mm in the drought condition. For control plants, root traits were almost constant during the experiment, whereas those in DS-8 and DS-12 treatments gradually changed. Differences between CK-8 and DS-8 treatments were detected in terms of root length, surface area, and volume. In particular, root length, surface area, and volume in DS-12 treatment of the minimum diametric class (0–1 mm) were always the lowest when compared with CK-12 and DS-8 treatments. There was more than 53.1%, with the maximum percentage in root length in DS-8 treatment (98.7%), in the frequency of root length, surface area, and volume in fine roots in drought-stressed plants.

Root vigor, RWC, proline, ABA, H₂O₂, and antioxidantase. Root vigor and RWC were significantly decreased (*P* < 0.05) by drought stress. They were 5.46 and 3.20 times higher in CK-12 treatment than in DS-12 treatment, respectively (Fig. 2A and B). The concentration of proline increased (*P* < 0.05) by 25.0% in DS-8 treatment than in CK-8 treatment, and no difference was observed among DS-8, CK-12, and DS-12 treatments (Fig. 2C). Compared with CK-8 treatment, ABA concentration was unchanged in DS-8 treatment. However, in DS-12 treatment, it increased significantly (*P* < 0.05) to 147.2 ng·g⁻¹ (Fig. 2D).

The imposing of drought resulted in significant H₂O₂, SOD, and ASA accumulations in *T. 406* roots. Compared with CK-8, they increased by 90.0%, 13.9%, and 35.5% (*P* < 0.05), respectively, in DS-8 plants. In DS-12 treatment, the concentration of H₂O₂ and activities of SOD and ASA increased significantly (Table 2).

Effect of drought stress on root tip ultrastructure. In CK plants, cells of *T. 406* plant roots had smooth and continuous cell membranes and cell walls (Fig. 3A). Mitochondria were easily recognized because of the wider and variable intracellular spaces between them. Besides, endoplasmic

Table 1. Root morphological parameters of *T. 406* plants under different treatments.

Treatment	Total length (m)	Surface area (cm ²)	Volume (cm ³)	SRL (m·g ⁻¹)	SRA (cm ² ·g ⁻¹)	Density (g·cm ⁻³)
CK-8	82.7 ± 9.1 a	1253 ± 22.1 a	22.9 ± 1.2 a	9.03 ± 1.0 a	137.6 ± 2.6 b	0.39 ± 0.04 c
DS-8	47.8 ± 3.6 b	726.5 ± 13.4 b	9.26 ± 1.1 b	3.39 ± 0.3 b	123.1 ± 3.2 b	0.63 ± 0.05 b
CK-12	86.2 ± 6.3 a	1193 ± 39.2 a	21.3 ± 2.1 a	9.79 ± 1.1 a	135.5 ± 4.3 b	0.41 ± 0.09 c
DS-12	23.6 ± 2.3 c	346.2 ± 19.3 c	4.37 ± 0.3 c	1.74 ± 0.3 c	56.75 ± 2.3 a	1.39 ± 0.07 a

Data are mean ± SE (*n* = 4). Different small letters within the same column are significantly different at *P* < 0.05. SRL = specific root length; SRA = specific root area.

Fig. 1. Total length (A), surface area (B), and volume (C) distribution at diametric classes of 0–1, 1–2, and >2 mm of *T. 406* plants under different treatments.

Fig. 2. Root vigor (A), relative water content (RWC, B), proline (C), and abscisic acid (ABA, D) of *T. 406* plants under different treatments. Data are mean $\pm$ SE ($n = 4$). Different lowercase letters indicate significant differences ($P < 0.05$).

Table 2. Content of H_2O_2 and activity of SOD and ASA in *T. 406* plant root under different treatments.

Treatment	H_2O_2 content ($\mu g \cdot g^{-1}$ FW)	SOD activity ($U \cdot g^{-1}$ FW)	ASA activity ($mg \cdot g^{-1}$ FW)
CK-8	2.0 ± 0.4 c	151.2 ± 11.1 c	26.7 ± 3.1 c
DS-8	3.6 ± 1.1 b	172.3 ± 10.6 b	36.2 ± 3.4 b
CK-12	2.1 ± 0.3 c	141.2 ± 9.1 c	25.2 ± 5.1 c
DS-12	4.7 ± 0.3 a	200.2 ± 15.3 a	42.3 ± 4.3 a

Data are mean $\pm$ SE ($n = 4$). Different small letters within the same column are significantly different at $P < 0.05$. H_2O_2 = hydrogen peroxide; SOD = superoxide dismutase; ASA = ascorbic acid; FW = fresh weight.

reticulae were well arranged (Fig. 3D). However, in DS-8 treatment, the organelle exhibited a dehydrated structure, and we can find a disrupted shape with dim membranes in distinct grana regions (Fig. 3B). Nucleus became considerably swollen. In addition, the envelopes of mitochondria were disrupted. The thylakoid-like organelle was loosened and distorted, beginning to disappear

(Fig. 3E). In DS-12 treatment, cells were clearly damaged because cell morphology showed obvious ultrastructural changes over time, and thus partitioning between organelle was lost because of drought (Fig. 3C). The main alterations included nucleus condensation and cytoplasm degeneration. In some instances, the cell walls became vesiculated (Fig. 3F).

Root nutrients and growth traits. TN concentration of *T. 406* roots in DS-12 treatment increased significantly ($P < 0.05$) compared with that in CK-12 and DS-8 treatments, respectively. The contents of TN were the least ($P < 0.05$) in DS-8 treatment, mainly attributing to the decreased root biomass and TN concentration (Table 3). TP concentration of *T. 406* roots, ranging between 3.8 and 4.2 $mg \cdot g^{-1}$, did not change significantly with soil drying. By contrast to root TN contents, there was no significant difference in TP contents in DS-8 and CK-12 plants (Table 3). At the end of this experiment, significant differences were detected between CK and DS treatments for root biomass, with that of CK-8 and CK-12 plants being $\approx 54.2\%$ and 44.2% higher ($P < 0.05$) than that of DS-8 and DS-12 plants, respectively. For root/shoot ratio, DS-12 plant was 24.4% ($P < 0.05$) and 7.1% higher than CK-12 and DS-8 plants, respectively (Table 3).

Discussion

Resources are often distributed inhomogeneously in the soil, and, thus, the morphology and physiology of roots are quite crucial for the absorption of soil water and nutrients. Varney and Canny (1993) reported that water absorbed by lateral roots accounted for more than 80% of the whole root system during the plant growth process. Before this experiment, few references were available describing root morphology and physiology of *Taxodium* and their relationships with water availability. Our results showed that root length, surface area, and volume of *T. 406* plants exhibited a decreasing pattern during progressive drought. Plant root traits are generally suppressed under dry conditions, and *T. 406* root biomass is sensitive to soil drying. This is a result of redistribution of the roots throughout the soil because basal regions of the parental roots are located in a limited space near the plant body (Ito et al., 2006). Under drought stress condition, a significant decrease in root volume would contribute to greater root density, leading to smaller mechanical impedance and greater penetrate capacity when *T. 406* plant roots are elongated. There is a consensus that stronger root vertical growth ability could maximize soil moisture capture and, thus, maintain a high plant water status under resource-limited conditions (Luo, 2010; Zhang et al., 2012). A similar result was obtained in a drought-resistant genotype: *Taxodium* hybrid ‘Zhongshanshan 118’ whose root/shoot ratio was increased significantly by progressive drought stress due to the relatively inhibited growth of leaf and stem (Shi et al., 2016).

Pemán et al. (2006) noted that lateral roots became thicker and consumed more energy during the vertical downward growth of taproots; thus, SRL and SRA may be used as a predictor of the benefit (length and area) to investment (DW) ratio of the root system. In DS-8 and DS-12 treatments, the

frequency of root length, surface area, and volume in fine roots increased, indicating a relatively high death rate of roots (>2 mm) in the drought condition, whereas SRL and SRA decreased compared with control treatments. This result indicated that *T. 406* plant root patterns generating in a water-deficit condition could be considered as more efficient than that observed in a well-water condition. Following the architecture named 'energy minimization' (McCulloh and Sperry, 2005), lateral roots in *T. 406* plants characterized by less massive roots per unit length or area were capable of maintaining a higher hydraulic conductance. Such responses in the lateral roots might be beneficial for transferring nutrients either from dead roots or aboveground parts (Ito et al., 2006). Evidenced by increased TN concentration and TP contents, the relatively high death rate of roots (>2 mm) in drought-stressed *T. 406* plants might help transfer N and P to fine roots.

Generally, plants undergo mild water stress after soil drying for 2–4 d, and then water stress becomes moderate after 6–10 d without irrigation and is considered severe after 12 d (Hsiao, 1973). Responses of plants to water deficit at the physiological and biochemical level involve various mechanisms, including those for minimizing water loss, scavenging toxic oxygen species, and

keeping cellular stability (Vanaja et al., 2011). In the present study, root RWC in drought-stressed *T. 406* plants decreased significantly, and the value of CK-12 treatment was 3.20 times of that in DS-12 treatment. Rampino et al. (2006) stated that water content in plants can be identified by measuring RWC at a particular time point. Thus, nonirrigation has caused part of water loss in *T. 406* roots in DS-8 and DS-12 treatments. Water deficit invariably leads to a considerable increase in the cellular antioxidant and then enhances reactive oxygen species, the most important of which is O_2^- . As the first threshold of enzymatic and nonenzymatic defense, SOD and ASA play a key role in catalyzing the dismutation of O_2^- into H_2O_2 . Proline accumulated at the same time to facilitate its hydroxyl groups bonding with hydrogen in water to stabilize critical enzymes and cellular structures (Jones, 1992). Compared with CK-8 *T. 406* plants, root SOD and ASA activity increased significantly in DS-8 plants and they kept increasing in DS-12 plants, with proline collaboratively protecting cytoplasmic proteins and cell membranes from desiccation. It has recently been found that the potential activation of *1-pyrroline-5-carboxylate synthetase* gene expression probably occurs with proline accumulation in *Taxodium* plants under drought stress conditions

(Wang et al., 2017). Similar reports of enzymatic and nonenzymatic defense to drought stress had been observed in roots of Kentucky bluegrass (*Poa pratensis*) and wheat (*Triticum aestivum*) (Bian and Jiang, 2009; Farooq et al., 2013). In addition, after being catalyzed from O_2^- , H_2O_2 was also reported to act as a signaling molecule, mediating environmental stress responses with ABA (Kong et al., 2016). It was suggested that ABA and H_2O_2 signaling is complicated, encompassing long-distance stomata closure and root hydraulic conductance (Sobotta et al., 2014; Zhang et al., 2006). Thus, pathways of accumulating ABA and H_2O_2 in *Taxodium* plants under drought stress require further confirmation.

Examining changes in root ultrastructure is usually regarded as a convenient and valuable approach to unveil the basic mechanisms under drought circumstance (Zhou et al., 2016). In our study, internal ultrastructure of cells in CK plants had smooth and continuous cell membranes and cell walls with typical mitochondria. However, the organelle structures of *T. 406* plant root tips were visibly damaged under drought stress. It is noteworthy that superimposed drought stress made nucleus swollen considerably. In DS-12 treatment, all the cells underwent dramatic changes, in which the organelles showed uncommon ultrastructural features of disorganization and disruption. So in *T. 406* plant lateral roots, reduced root biomass and activity (as TTC-reducing capacity) were mainly due to dramatic damages at the ultrastructural level. Other reports showed the close relationship between cellular damage and biomass change (Jin et al., 2010; Zhou et al., 2016).

Conclusion

The present study provides substantial information about lateral root traits of *T. 406* plants under drought stress. Our result implies that the elongation of lateral roots was sensitive to soil drying, and the benefit-to-investment ratio was reduced to enhance the uptake of water and nutrients in response to drought. Meanwhile, water deficit decreased root vigor by causing cellular damages with enhancement in enzymatic and nonenzymatic activities. These traits enable *T. 406* plants to survive in xeric environments. Therefore, selection of *Taxodium* hybrids with plastic root morphology is strongly recommended in the future breeding of drought-resistant varieties.

Fig. 3. Effect of drought stress on ultrastructure ($\times 500$, A–C; $\times 2000$, D–F) of *T. 406* plant root tips. (A and D) Root sample of control; (B and E) root sample of DS-8; (C and F) root sample of DS-12. CW = cell wall; ER = endoplasmic reticulum; M = mitochondrion; N = nucleus; V = vacuole.

Table 3. Root nutrients and growth traits of *T. 406* plants under different treatments.

Treatment	Concn (mg·g ⁻¹ DW)		Content (mg)		Aboveground DW (g)	Root DW (g)	Root/shoot ratio
	TN	TP	TN	TP			
CK-8	15.4 ± 3.9 b	4.2 ± 0.6 a	140 ± 7.6 a	38.2 ± 2.6 a	25.0 ± 0.2 a	9.1 ± 0.6 a	0.36 ± 0.02 b
DS-8	14.6 ± 2.4 b	3.8 ± 0.9 a	86.1 ± 7.7 b	22.4 ± 1.9 b	14.0 ± 0.2 b	5.9 ± 0.4 b	0.42 ± 0.04 ab
CK-12	16.4 ± 2.9 b	3.9 ± 0.6 a	144 ± 7.6 a	34.3 ± 2.1 a	25.8 ± 0.3 a	8.8 ± 0.6 a	0.34 ± 0.04 b
DS-12	24.3 ± 1.7 a	4.0 ± 0.7 a	148 ± 10.6 a	24.4 ± 3.3 b	13.6 ± 0.3 b	6.1 ± 0.3 b	0.45 ± 0.04 a

Data are mean ± SE (n = 4). Different small letters within the same column are significantly different at $P < 0.05$. TN = total nitrogen; TP = total phosphorus; DW = dry weight.

Literature Cited

- Allen, C.D., A.K. Macalady, H. Chenchouni, D. Bachelet, N. McDowell, M. Vennetier-Kitzberger, T. Rigling, D.D. Breshears, E.T. Hogg, G. Patrick, R. Fensham, Z. Zhen, C. Jorge, D. Natalia, L. Jong-Hwan, A. Gillian, W. Steven, S. Akkin, and C. Neil. 2010. A global overview of drought and heat-induced tree mortality reveals emerging climate change risks for forests. *For. Ecol. Mgt.* 259:660–684.
- Bates, L., R. Waldren, and I. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207.
- Bian, S. and Y. Jiang. 2009. Reactive oxygen species, antioxidant enzyme activities and gene expression patterns in leaves and roots of *Kentucky bluegrass* in response to drought stress and recovery. *Scientia Hort.* 120:264–270.
- Cavelier, J. 1992. Fine-root biomass and soil properties in a semideciduous and a lower montane rain forest in Panama. *Plant Soil* 142 (2):187–201.
- Creech, D., L. Zhou, Y. Yunlong, and T. Eguiluz-Piedra. 2011. Can *Taxodium* be improved? *Arnoldia* 69:11–20.
- Diffenbaugh, N.S. and M. Scherer. 2011. Observational and model evidence of global emergence of permanent, unprecedented heat in the 20th and 21st centuries. *Clim. Change* 107:615–624.
- Doussan, C., G. Vercambre, and L. Pagès. 1999. Water uptake by two contrasting root systems (maize, peach tree): Results from a model of hydraulic architecture. *Agronomie* 19:255–263.
- Farooq, M., M. Irfan, T. Aziz, I. Ahmad, and S.A. Cheema. 2013. Seed priming with ascorbic acid improves drought resistance of wheat. *J. Agron. Crop Sci.* 199:12–22.
- Hardie, M., B. Clothier, S. Bound, G. Oliver, and D. Close. 2014. Does biochar influence soil physical properties and soil water availability? *Plant Soil* 376(1–2):347–361.
- Hartmann, H., H.D. Adams, W.R.L. Anderegg, S. Jansen, and M.J.B. Zeppel. 2015. Research frontiers in drought-induced tree mortality: Crossing scales and disciplines. *New Phytol.* 205:965–969.
- Hsiao, T.C. 1973. Plant responses to water stress. *Annu. Rev. Plant Physiol.* 24:519–570.
- Ito, K., K. Tanakamaru, S. Morita, J. Abe, and S. Inanaga. 2006. Lateral root development, including responses to soil drying, of maize (*Zea mays*) and wheat (*Triticum aestivum*) seminal roots. *Physiol. Plant.* 127:260–267.
- Jin, J., G. Wang, X. Liu, L. Mi, Y. Li, Y. Xu, and S.J. Herbert. 2010. Genetic improvement of yield shapes the temporal and spatial root morphology of *soybean* (*Glycine max*) grown in north-east China. *N. Z. J. Crop Hort. Sci.* 38:177–188.
- Jones, H. G. 1992. *Plants and microclimate: A quantitative approach to environmental plant physiology.* Cambridge Univ. Press.
- Kano, M., Y. Inukai, H. Kitano, and Y. Akira. 2011. Root plasticity as the key root trait for adaptation to various intensities of drought stress in rice. *Plant Soil* 342(1–2):117–128.
- Kirk, P.L. 1950. Kjeldahl method for total nitrogen. *Anal. Chem.* 22(2):354–358.
- Kong, X., Z. Luo, H. Dong, A.E. Eneji, and W. Li. 2016. H₂O₂ and ABA signaling are responsible for the increased Na⁺ efflux and water uptake in *Gossypium hirsutum* L. roots in the non-saline side under non-uniform root zone salinity. *J. Expt. Bot.* 67:2247–2261.
- Liu, R.X., Z.G. Zhou, W.Q. Guo, B.L. Chen, and D.M. Oosterhuis. 2008. Effects of N fertilization on root development and activity of water-stressed cotton (*Gossypium hirsutum* L.) plants. *Agr. Water Mgt.* 95:1261–1270.
- Luo, L.J. 2010. Breeding for water-saving and drought-resistance rice (WDR) in China. *J. Expt. Bot.* 61:3509–3517.
- McCulloh, K.A. and J.S. Sperry. 2005. Patterns in hydraulic architecture and their implications for transport efficiency. *Tree Physiol.* 25:257–267.
- Mel'Tser, R.A. 1960. Colorimetric method of determining phosphorus in soils and plants. *Pochvovedenie* 6:103–106.
- Pemán, J., J. Voltas, and E. Gil-Pelegrin. 2006. Morphological and functional variability in the root system of *Quercus ilex* L. subject to confinement: Consequences for afforestation. *Ann. For. Sci.* 63:425–430.
- Rampino, P., S. Pataleo, C. Gerardi, G. Mita, and C. Perrotta. 2006. Drought stress response in wheat: Physiological and molecular analysis of resistant and sensitive genotypes. *Plant Cell Environ.* 29:2143–2152.
- Rouphael, Y., M. Cardarelli, G. Colla, and E. Rea. 2008. Yield, mineral composition, water relations, and water use efficiency of grafted mini-watermelon plants under deficit irrigation. *HortScience* 43:730–736.
- Rowland, L. 2015. Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature* 528:119–122.
- Sharma, D.K., A. Dubey, M. Srivastav, A. Singh, R. Sairam, R.A. Pandey-Dahuja, and C. Kaur. 2011. Effect of putrescine and paclobutrazol on growth, physiochemical parameters, and nutrient acquisition of salt-sensitive citrus rootstock Karna khatta (*Citrus karna* Raf.) under NaCl stress. *J. Plant Growth Regulat.* 30:301–311.
- Shi, Q., Y. Yin, Z. Wang, W. Fan, and J. Hua. 2016. Physiological acclimation of *Taxodium* hybrid 'Zhongshanshan 118' plants to short-term drought stress and recovery. *HortScience* 51:1159–1166.
- Sobotta, M.C., W. Liou, S. Stöcker, S. Sarah, T. Deepti, O. Michael, R. Thomas, N.D.S. Annette, and P.D. Tobias. 2014. Peroxiredoxin-2 and STAT3 form a redox relay for H₂O₂ signaling. *Nat. Chem. Biol.* 11(1):64–70.
- Vanaja, M., S.K. Yadav, G. Archana, N.J. Lakshmi, P.R.R. Reddy, P. Vagheera, S.K.A. Razak, M. Maheswari, and B. Venkateswarlu. 2011. Response of C4 (maize) and C3 (sunflower) crop plants to drought stress and enhanced carbon dioxide concentration. *Plant Soil Environ.* 57(5):207–215.
- Varney, G. and M.J. Canny. 1993. Rates of water uptake into the mature root system of maize plants. *New Phytol.* 123:775–786.
- Wang, Z., F. Yu, Q. Shi, J. Hua, L. Xuan, W. Fan, and Y. Yin. 2017. Cloning and expression analysis of *THP5CS* and *THA-OAT* gene in *Taxodium* hybrid 'Zhongshanshan 407' under drought stress. *J. Nanjing For. Univ.* 41 (5):191–196 (in Chinese).
- Wu, X., W. Xiaoling, S. Wanping, and M. Guicheng. 2016. Preliminary afforestation evaluation of *Taxodium* Zhongshanshan "405, 406, 407" in Rudong county seawall. *J. Fujian For. Sci. Technol.* 43(4):62–65 (in Chinese).
- Zhang, H., C. Ju, T. Chen, Z. Cao, Z. Wang, and J. Yang. 2012. Effect of water-saving irrigation on the grain yield of water-saving and drought-resistance rice and its physiological bases. *Sci. Agr. Sin.* 45:4782–4793 (in Chinese).
- Zhang, J., W. Jia, J. Yang, and A.M. Ismail. 2006. Role of ABA in integrating plant responses to drought and salt stresses. *Field Crops Res.* 97:111–119.
- Zhou, Q., Y. Wu, Z. Chonglan, X. Xing, L. Liu, H. Jiang, and H. Xing. 2016. Triadimefon induced C and N metabolism and root ultra-structural changes for drought stress protection in soybean at flowering stage. *J. Plant Growth Regulat.* 35:222–231.