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ORIGINAL PAPER

Salicylic acid induces physiological and biochemical changes in *Torreya grandis* cv. *Merrillii* seedlings under drought stress

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Abstract

Key message **SA treatment effectively ameliorated the negative effect of moderate drought stress on *T. grandis* Seedlings through increasing the water content, Pn, proline content, antioxidant enzymes activity and reducing MDA.**

Abstract Water availability is one of the most critical factors that limits the growth and development of plants. Salicylic acid (SA) is an important signal molecule that modulates plant responses to abiotic stress. To elucidate the regulating mechanism of exogenous SA on *Torreya grandis* cv. *Merrillii* under different water stresses, a pot experiment was conducted in a greenhouse. Exposure of *T. grandis* seedlings to drought conditions resulted in reduced growth rate that was associated with a decline in water content and CO_2 assimilation. Foliar application of SA effectively increased the water content, net CO_2 assimilation rate, proline content and antioxidant enzymes activity in the plants, which helped *T. grandis* to acclimate to moderate drought stress and increase the shoot dry matter. However, when the plants were under severe drought stress, the relative water content and CO_2 assimilation in the SA-treated plants were significantly lower than those in the control plants. Therefore, our results indicated that SA can effectively ameliorate the negative effect of moderate drought stress on *T. grandis* seedling growth.

Keywords *Torreya grandis* cv. *Merrillii* · Salicylic acid · Water stress · Osmotic adjustment system

Abbreviations

AOS	Active oxygen species
CAT	Catalase
Ci	The intercellular CO_2
Gs	Stomatal conductance
MDA	Malondialdehyde
POD	Peroxidase
Pn	Net CO_2 assimilation rate
REC	Relative electrolyte conductivity
RWC	Relative water content
SA	Salicylic acid
SOD	Superoxide dismutase
Tr	Transpiration rate

Introduction

Genus *Torreya* is a primitive member of the yew family (Taxaceae). Six species of this genus are currently known worldwide. Among these, *Torreya jackii,* and *Torreya grandis* are endemic in China; *Torreya nucifera* grows in Japan and Korea, and *Torreya californica* and *Torreya*

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taxifolia grow in California and western Florida, respectively (Kang and Tang 1995). *T. jackii, T. nucifera, T. californica* and *T. taxifolia* are now included in the IUCN (International Union for Conservation of Nature) Red List of Threatened Species (http://www.iucnredlist.org/), while *T. fargesii* and *T. grandis* have been listed as national second-grade key protected wild plants (Yu 1999). The conservation and recovery of those species have received increasing attention in recent years. *Torreya grandis* cv. *Merrillii*, a tree species with significant economic value, has drupe-like fruits with nut-seeds that have been used as food for 1,000 years in China. In China, it is also an indigenous medicinal plant due to its anthelmintic, antitussive, carminative, laxative, antifungal, antibacterial and antitumor activity (Huang et al. 2001). *T. grandis* is an important economic tree species in southern China and is mostly planted on hills and mountain slopes without artificial irrigation system. This part of China has usually been suffering from serious droughts from summer to autumn, with a precipitation of only 120 mm between July and October, which is only 30 % of the normal year although most of the annual precipitation in the region is 1,000–1,500 mm (Zhou et al. 2003; Feng and Hong, 2007). The growth and development of these tree seedlings is strongly limited by water stress during the dry season, leading to the decrease of vegetative and reproductive growth. Therefore, it is necessary to investigate effects of water stress on the growth of *T. grandis* seedlings.

Water stress is one of the most important environmental factors that can regulate plant growth and development. It limits plant production, especially for plant species that are presently endangered, and has adverse effects on plant photosynthesis, growth and yield (Cornic and Massacci 1996). Osmotic adjustment in terms of accumulating compatible solutes (proline) has been considered as an important physiological adaptation for plants to resist drought (Morgan 1984), which facilitates the extraction of water from dry soils, thereby maintaining cell turgor, gas exchange and growth under very dry environments (White et al. 2000). Increasing evidence suggests that drought induces oxidative stress through the formation of active oxygen species (AOS) (Asada 1999), which is due to stomatal closure (Ozkur et al. 2009). AOS could damage many important cellular components in plants such as lipids, proteins, photosynthetic pigments and enzymes (Smirnoff 1993). In order to overcome oxidative stress, plants have developed enzymatic antioxidant systems (SOD, CAT, POD) that scavenge AOS and stabilize photosynthetic complexes (Verhagen et al. 2004). Increasing evidence suggests that SA can act as an important signaling molecule and has diverse effects on tolerance to biotic and abiotic stresses (Senaratna et al. 2000). Exogenous application of SA may also influence a range of development and physiological process, such as water relation (Ying et al. 2013), antioxidant enzymes (Janda et al. 1999; Hayat et al. 2008, 2010), photosynthetic pigment (Rajasekaran and Blum 1999), PSII function (Wang et al. 2010), Rubisco (Singh and Usha 2003) and plant growth (Bandurska and Cieślak 2012; Gutiérrez-Coronado et al. 1998; Sakhabutdinova et al. 2003).

In the past years, numerous studies have been focused on *T. grandis* species distribution and its ecological characteristics (Cheng et al. 2007), endophytic fungi (Li et al. 1998; Huang et al. 2001), chemical constituents (Saeed et al. 2007), the seed fatty acid composition and its distribution (Wolff et al. 1998) and therapeutic use of extract of the seed (Chen et al. 2006). However, little attention has been paid to the physiological responses of *T. grandis* to SA under water stress. Although selection and breeding are the ultimate means to produce drought-tolerant crop plants, exogenous application of osmoprotectants, growth promoters and antioxidant compounds to plants has been considered a short-term solution to ameliorate the adverse effects of drought stresses on plants (Singh and Usha 2003). Therefore, this study aimed to investigate whether salicylic acid can effectively ameliorate the negative effects of drought stress on *T. grandis* seedlings growth. A better understanding of the effects of drought on *T. grandis* is vital for improved management practices and breeding efforts in agriculture and for the conservation and recovery of the endangered plant species, *T. grandis*, in the future.

Materials and methods

Plant material and growth conditions

The experiments using plastic pots under different drought stress treatments were conducted in the controlled environment room of Zhejiang A & F University (N30°23′, E119°72′), China in 2012. In practice, 2-year-old seedlings are usually used for afforestation, and the growth of 2-year seedlings makes a significant influence on the establishment of *T. forests*. Therefore, we chose 2-year-old seedlings as the experimental materials. In late March, 2-year-old healthy and homogenous *T. grandis* seedlings were transferred to plastic pots (13.5 cm inner-diameter, 16 cm height, with holes in the bottom, one seedling per pot) filled with 4 kg of the mixture of silt and perlite (3:1 v/v, pH 6.40) soil. The soil is a loam with organic matter content 2.90 %, total N content 0.255 %, total P content 0.125 %, and total K content 1.574 %. All the pots were irrigated daily to keep well watered and maintained at 75 % field capacity of soil. New shoots of *T. grandis* begin to emerge

during April to June. Therefore, 8 weeks later, a completely randomized design with three replications per treatment and five plants per replication (plastic pot) was adopted. Five treatments were as follows: (T1) Control (watered and maintained at 75–80 % field capacity); (T2) Moderate water stress (watered and maintained at 50–55 % field capacity); (T3) Moderate water stress + SA (treated with 0.5 mM SA and maintained at 50–55 % field capacity), (T4) Severe water stress (watered and maintained at 35–40 % field capacity); and (T5) Severe water stress + SA (treated with 0.5 mM SA and maintained at 35–40 % field capacity). During the experiment, the pots were re-watered to different field capacities by replacing the amount of water transpired every day. SA was dissolved in ethanol, and then added to "Tween-20" (0.1 % dilute solution) to facilitate spreading of the solution on the plant-leaf surface. The desired concentration of SA was reached, using double distilled water (DDW). SA was sprayed on leaves twice daily, at 0700 and 1800 hours, beginning 3 days before water stress treatment. SA was sprayed on both the adaxial and abaxial surfaces in a leaf until dripping by using a common insecticidal atomizer. The control was sprayed with SA or distilled water but there was no difference between them in the pre-experiment (data not shown). Thus, the control was sprayed with distilled water in this study.

Thirty days after treatments, the fresh leaves were collected from plants of each drought treatment and cleaned with moistened cloth to remove any surface contamination, and then immediately frozen in liquid nitrogen and stored at −70 °C. 0.3 g of frozen leaves was ground at 4 °C in a mortar with 8 mL of 50 mM phosphate buffer solution (pH 7.8) containing 1 % polyethylene pyrrole (PVP). The homogenate was centrifuged at 10,000 rpm at 4 °C for 15 min. Supernatant was collected for measuring enzyme activities.

Analysis of growth and biomass

Sixty days after treatments, one intact plant (aboveground shoot and underground root) from each replicate of different drought treatment was harvested for biomass analyses. After drying to constant weight at 60 °C dry weights were determined.

Proline content determination

Proline accumulation was determined using the method described by Bates et al. (1973) with slight modifications. Approximately 0.2 g of fresh leaf material was homogenized in 5 ml of 3 % aqueous sulphosalicylic acid. Samples were mixed, heated in boiling water for 30 min and filtered through Whatman No. 2 filter paper. 2 ml of the filtrate was mixed with 2 ml acid-ninhydrin and 2 ml glacial acetic acid in a test tube. The mixture was placed in a water bath for 1 h at 100 °C and cooled to room temperature. The reaction mixture was extracted with 5 ml toluene and the chromophore containing toluene was aspirated. The absorbance was measured at 520 nm using toluene for a blank. The proline concentration was calculated using proline standards (0–50 mg ml^{-1}) in an identical manner.

Leaf water content determination

Leaves were collected from different water-treated plants to determine relative water content (RWC). The RWC was calculated as the following formula:

$$\mathrm{RWC}\ (\%) = [(\mathrm{Wf} - \mathrm{Wd}) / (\mathrm{Ws} - \mathrm{Wd})] \times 100$$

0.5 g fresh leaves were immediately weighed (fresh weight, Wf), after rehydrating for 24 h at dark (saturated weight, Ws) and then oven-dried at 85 °C for 24 h to constant mass (Wd).

Lipid peroxidation measurement

The lipid peroxidation level was determined in terms of malondialdehyde (MDA) contents by the method of Zheng et al. (2006). Fresh leaves (1.0 g) was ground in 10 % trichloroacetic acid and then centrifuged at 3,000×*g* for 10 min. To each 2 mL aliquot of the supernatant, 2 mL of 0.6 % thiobarbituric acid in 10 % TCA was added. The mixtures were heated at 100 °C for 15–30 min and then quickly cooled in an ice bath. After centrifugation at 5,000×*g* for 20 min, the absorbance of the supernatant was recorded at 532, 600 and 450 nm. Lipid peroxidation was expressed as μmol g^{-1} by using the following formula: $6.45(A_{532} - A_{600}) - 0.56A_{450}$.

Detection of relative electrolyte conductivity

Membrane permeability was estimated by measuring the leaf relative electrolyte conductivity following the protocol described by Nayyar (2003). 0.2 g discs were briefly rinsed with deionized water and immersed in a test tube with 30 mL deionized water for 12 h. Then electrical conductivity (initial EC) of the solution was measured with a conductivity meter (Model DJS-1C; Shanghai Analytical Instrument Co. Shanghai, China). Then the samples were heated at 100 °C for 20 min and conductivity (final EC) in the bathing solution was read again.

$$\text{Membrane permeability was calculated as EC}(\%) = \left(\frac{\text{initial EC}}{\text{final EC}}\right) \times 100$$

Chlorophyll concentration determination

About 0.1 g of finely cut and well-mixed samples were repeatedly extracted with 8 mL 95 % acetone. Chlorophyll was extracted at 4 °C for 24 h in darkness and shaken three or four times until they were blanched. The absorbance was measured with a spectrophotometer Shimadzu UV-2550 (Kyoto, Japan) at 646 and 663 nm after centrifugation of the mixture on standing. Chlorophyll concentrations were calculated by the standard method of Arnon (1949) and expressed in mg/g fresh weight (FW).

Analysis of gas exchange

The youngest healthy fully developed leaves randomly selected from one branch were chosen for gas exchange measurements, which were measured on annual shoot attached using a portable photosynthesis measuring instrument (LI-6400, LiCor, Inc. Lincoln, NE, USA) equipped with an 6400-05 conifer chamber at a concentration of 400 μmol mol^{-1} CO_2 and 50 % relative humidity with natural light source. The temperature of chamber was kept at 29–32 °C.

Determination of antioxidant enzyme activities

Activity of Superoxide Dismutase (SOD) was assayed by monitoring its ability to inhibit the photochemical reduction of NBT (Beauchamp and Fridovich 1971). One unit of SOD was defined as the amount of enzyme necessary to inhibit the reduction of cytochrome c by 50 %. Catalase (CAT) activity was measured by monitoring the disappearance of H_2O_2 (Fu and Huang 2001). This can be detected by measuring the decrease in absorbance at 240 nm of a reaction mixture consisting of 1.5 mL 50 mM sodium phosphate buffer (pH 7.8), 0.3 mL 100 mM H_2O_2 and 0.2 mL enzyme extract and One CAT unit is defined as the amount of enzyme necessary to decompose 1 mmol min^{-1} H_2O_2 under the above-mentioned assay conditions. Peroxidase (POD) activity in leaves was estimated using the method of Fu and Huang et al. (2001). Peroxidase was assayed using guaiacol as the substrate.

Statistical analysis

The LSD test and analysis of variance (ANOVA, GLM procedure) were performed using SAS 9.2 Institute Inc. (1999) software. Simple correlation analysis was carried out using the SAS program to determine the relationship between each physiological variable. The data are presented as the mean ± SD. Differences at $P < 0.05$ were considered significant.

Table 1 The effects of SA on dry matter of shoot and root in *T. grandis* grown under drought treatments (Mean ± SD; $n = 3$)

Treatments	Shoot (*g*)	Root (*g*)
T1	25.00 ± 0.82^{a}	13.56 ± 1.03^{ab}
T2	20.35 ± 1.20^{c}	11.43 ± 0.79^{c}
T3	22.71 ± 1.42^{b}	14.00 ± 0.22^{a}
T4	19.66 ± 1.22^{c}	11.14 ± 1.01^{c}
T5	20.74 ± 1.12^{bc}	12.19 ± 0.66^{bc}

Drought treatments: T1, control; T2, Moderate water stress; T3, Moderate water stress + 0.5 mM SA; T4, Severe water stress; T5, Severe water stress + 0.5 mM SA

Letters indicate statistical differences ($P < 0.05$) according to an LSD test; same letter denotes no significant difference among treatments

Results

Dry mass of shoot and root of *T. grandis* in different water stress and SA treatments

Exposure of plants to water deficit for 60 days led to a decrease in both dry mass of shoot and root by 18.6 % ($P < 0.05$) and 15.7 % ($P < 0.05$) respectively under moderate water stress, while 21.4 % ($P < 0.05$) and 17.8 % ($P < 0.05$) under severe water stress (Table 1). SA-treated plants had significantly higher dry mass of shoot and root than the plants without SA under moderate water stress. Under the moderate water stress, dry mass of shoot and root in SA-treated plants increased by 11.6 % ($P < 0.05$) and 22.5 % ($P < 0.05$) in comparison to non SA-treated plants. However, no significant differences were found in dry mass of shoot and root between treated with and without SA treatments under severe water stress.

Relative water content and proline content of *T. grandis* in different water stress and SA treatments

Relative water content is useful variable to evaluate the physiological water status of plants (González and González-Vilar 2003). In comparison with well-watered plants, RWC decreased by 8.16 % ($P < 0.05$) and 16.10 % ($P < 0.05$) in moderate and severe water-stressed plants, respectively. Water stress dramatically induced the accumulation of proline in leaves. Under water deficit conditions, spraying SA did not significantly change RWC. However, there was no significant difference in RWC between SA-treated plants under moderated water stress and well-watered plants (Fig. 1). Proline content in SA-treated leaf was much higher than that in non SA-treated leaf. With SA treatments, proline in moderate and severe water-stressed plants increased by 28.4 % ($P < 0.05$) and 16.6 % ($P < 0.05$), respectively.

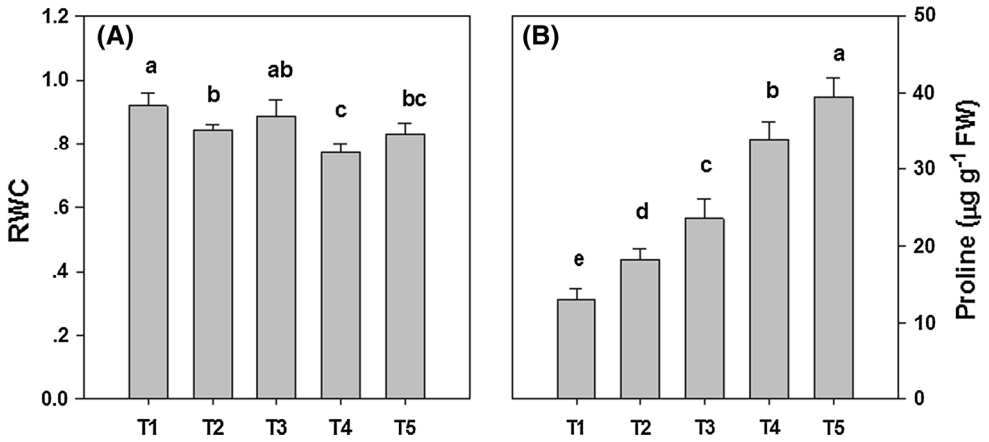

Fig. 1 Influence of salicylic acid treatments on the **a** relative water content (RWC) **b** proline content in *T. grandis* grown under water stress. Drought treatments: T1, control; T2, Moderate water stress; T3, Moderate water stress + 0.5 mM SA; T4, Severe water stress; T5, Severe water stress + 0.5 mM SA. *Letters* indicate statistical differences ($P < 0.05$) according to an LSD test; the *same letter* denotes no significant difference among treatments, $n = 3$

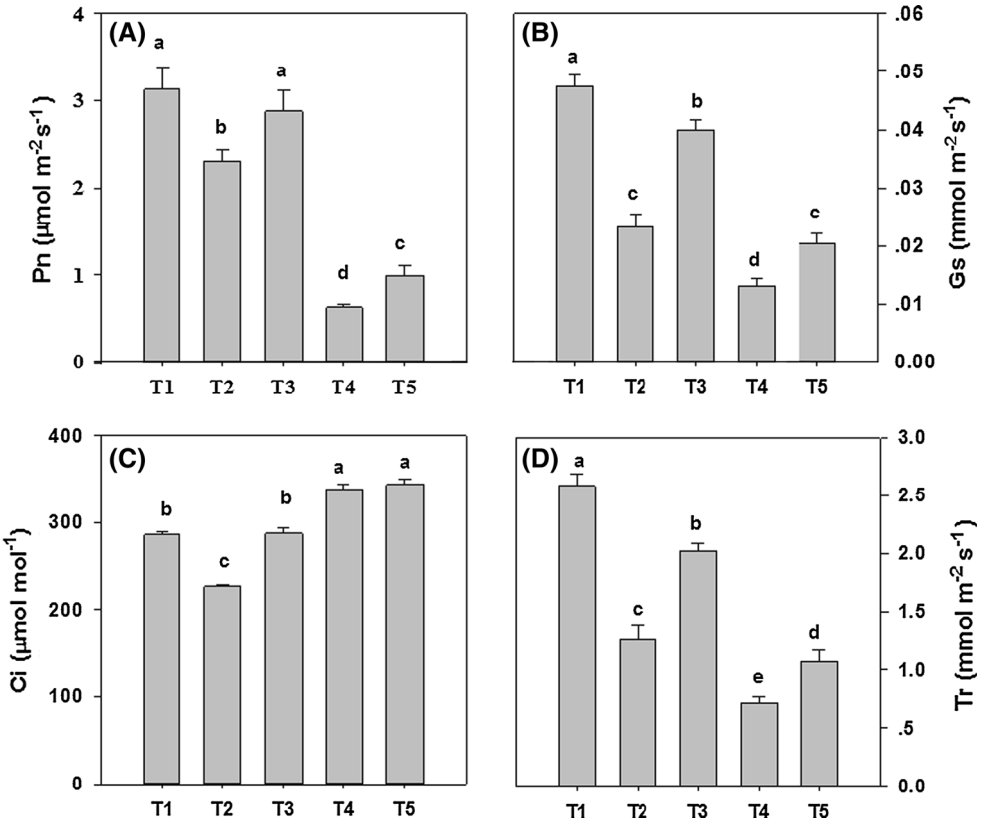

Fig. 2 Influence of salicylic acid treatments on the **a** net photosynthetic rate (Pn) **b** stomatal conductance (Gs), **c** internal carbon dioxide concentration (Ci) and **d** transpiration rate (Tr) in *T. grandis* grown under water stress. Drought treatments: T1, control; T2, Moderate water stress; T3, Moderate water stress + 0.5 mM SA; T4, Severe water stress; T5, Severe water stress + 0.5 mM SA. *Letters* indicate statistical differences ($P < 0.05$) according to an LSD test; the *same letter* denotes no significant difference among treatments, $n = 3$

Gas exchange parameters of *T. grandis* in different water stress and SA treatments

Water deficit reduced net assimilation rate (Pn), stomatal conductance (Gs) and transpiration rate (Tr) of leaves. As compared with control, Pn, Gs and Tr dramatically decreased by 26.16 % ($P < 0.05$), 51.06 % ($P < 0.05$) and 50.74 % ($P < 0.05$) under moderate drought stress, and by 79.92 % ($P < 0.05$), 72.34 % ($P < 0.05$) and 72.42 % ($P < 0.05$) under severe drought stress, respectively (Fig. 2a, b, d). As for the intercellular CO_2 (Ci), it was significantly decreased under moderate water stress, but was increased under severe drought stress (Fig. 2c). SA treatment caused increases in Pn, Gs, Tr in leaves under water stress. Gs in SA-treated plants increased by about 71.4 % ($P < 0.05$) and 56.8 % ($P < 0.05$) than that in non-

Table 2 The effects of SA on Chl ($a + b$) and Chl (a/b) in *T. grandis* grown under water stress (Mean ± SD; $n = 3$)

Treatment	Chl ($a + b$) (mg g^{-1}FW)	Chla/b
T1	1.239 ± 0.023[a]	3.318 ± 0.002[a]
T2	1.010 ± 0.067[b]	3.710 ± 0.306[a]
T3	1.229 ± 0.036[a]	3.312 ± 0.062[a]
T4	0.852 ± 0.144[c]	4.647 ± 1.918[a]
T5	1.135 ± 0.070[ab]	3.374 ± 0.056[a]

Drought treatments: T1, control; T2, Moderate water stress; T3, Moderate water stress + 0.5 mM SA; T4, Severe water stress; T5, Severe water stress + 0.5 mM SA

Letters indicate statistical differences ($P < 0.05$) according to an LSD test, same letter denotes no significant difference among treatments, $n = 3$

SA-treated plants under moderate and severe water stress, respectively. Ci in SA- treated plants under moderate water stress was similar to that in well-watered plants. However, Ci in SA-treated or non SA-treated plants under severe water stress was significant higher than that in well-watered plants.

Photosynthetic pigments content of *T. grandis* in different water stress and SA treatments

Chlorophyll is a biologically important pigment, which is utilized for photosynthetic conversion of inorganic molecules or ions to organic bio-molecules. With the increasing of water stress degree, Chl ($a + b$) contents significantly decreased. Compared with control, Chl ($a + b$) concentration decreased by 18.48 % ($P < 0.05$) and 31.23 % ($P < 0.05$) under moderate and severe water stress, respectively (Table 2). However, SA application to water-stressed plants significantly increased chlorophyll content. As compared with non-SA treatment, SA treatment increased Chl ($a + b$) by 21.68 % ($P < 0.05$) and 33.22 % ($P < 0.05$) under moderate and severe water stress, respectively. There were no significant differences in Chla/b ratio ($P > 0.05$) among the different water treatments.

Antioxidant enzymes and cellular damages of *T. grandis* in different water stress and SA treatments

As showed in Fig. 3, the activities of peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activities in *T. grandis* seedlings were significantly affected by drought and SA treatment. Under moderate water stress, SOD and CAT activities increased by 23.71 % ($P < 0.05$) and 24.10 % ($P < 0.05$), respectively. Severe water stress induced higher SOD and CAT but lower POD activity than moderate water stress. The response of SOD, CAT and

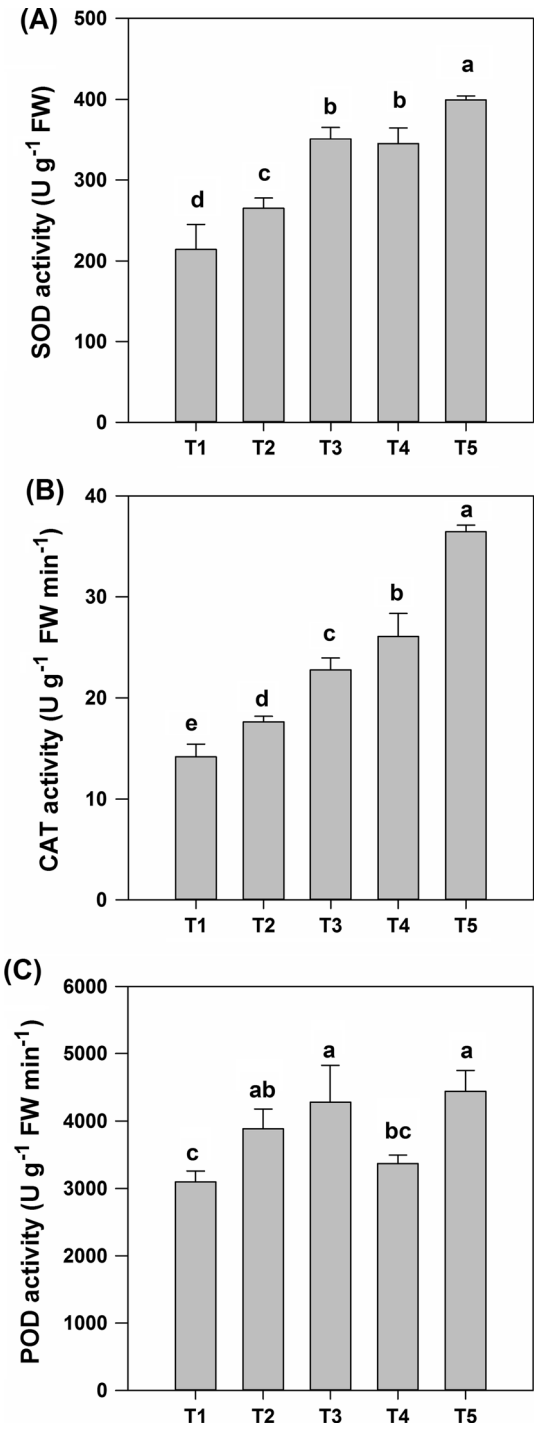

Fig. 3 Influence of salicylic acid treatments on the **a** SOD, **b** CAT and **c** POD in *T. grandis* grown under water stress. Drought treatments: T1, control; T2, Moderate water stress; T3, Moderate water stress + 0.5 mM SA; T4, Severe water stress; T5, Severe water stress + 0.5 mM SA. *Letters* indicate statistical differences ($P < 0.05$) according to an LSD test; the *same letter* denotes no significant difference among treatments, $n = 3$

POD activities in *T. grandis* seedlings to SA treatment was similar. SA treatment induced the increases in SOD, POD and CAT activity under water stress.

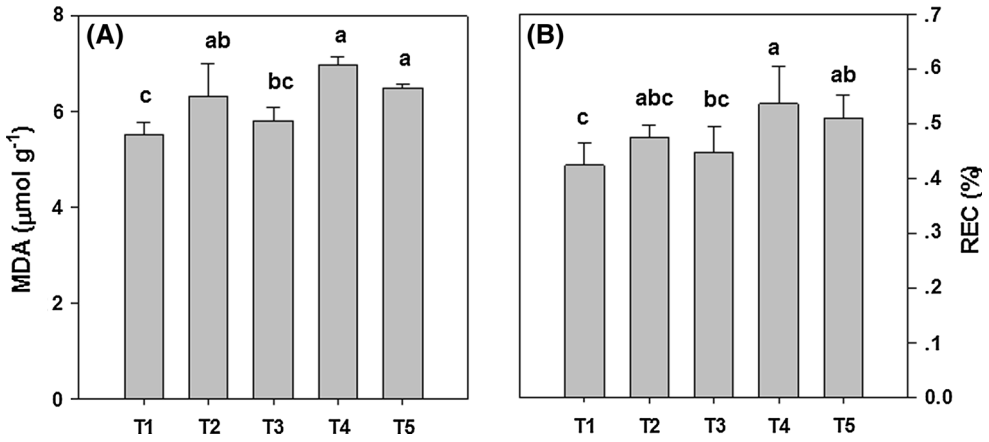

Fig. 4 Influence of salicylic acid treatments on the **a** malondialdehyde (MDA), **b** relative electrolyte conductivity (REC) in *T. grandis* cv. *Merrillii* grown under water stress. Drought treatments: T1, control; T2, Moderate water stress; T3, Moderate water stress + 0.5 mM SA; T4, Severe water stress; T5, Severe water stress + 0.5 mM SA. *Letters* indicate statistical differences ($P < 0.05$) according to an LSD test; the *same letter* denotes no significant difference among treatments, $n = 3$

Compared with control, moderate and severe drought increased MDA content by 14.49 % ($P < 0.05$) and 26.09 % ($P < 0.05$), REC by 11.90 % ($P > 0.05$) and 26.38 % ($P < 0.05$), respectively (Fig. 4). To some extent, SA treatments prevented lipid peroxidation of membrane. Compared with non SA-treated plants, the reduction in MDA in SA-treated seedlings was about 8.2 % ($P > 0.05$), under moderate water stress and 6.8 % ($P > 0.05$) under severe stress, respectively.

Discussion

Under conditions of water stress, plants sense changes in water availability and trigger a set of plant adaptive responses, including control of water status and carbon uptake to increase water uptake and keep plant growth (Hayat et al. 2008). Plants grown in water deficit conditions had reduced biomass accumulation, CO_2 assimilation and activities of photosynthesis-related enzymes accompanied with increased H_2O_2 accumulation, lipid peroxidation and altered antioxidant enzymes. Numerous studies indicated that plants could enhance SA to cope with low water availability by inducing a series of biochemical, physiological and molecular adaptation strategies (Bandurska and Cieślak 2012). In this study, we intended to demonstrate whether SA can induce a set of special physiological responses including responses of dry matter, RWC, proline, photosynthesis, photosynthetic pigments, antioxidant enzyme activities and MDA, REC that enabled *T. grandis* to better alleviate water stress.

Drought usually occurs in southern China from summer to autumn (Zhou et al. 2003; Feng and Hong 2007), which is the main environmental factor limiting plant productivity (Cornic and Massacci 1996). In this study, a marked reduction in growth of both shoot and root of *T. grandis* seedling was observed due to drought stress (Table 1). Such a reduction in growth may be an adaptive response to water stress, as it was observed in many other plants species (Degu et al. 2008; Efeoğlu et al. 2009). Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells (Nonami 1998). Consequently, the reduction in *T. grandis* seedling growth is a typical symptom under water stress.

Several studies have supported the notion of a major role of SA in modulating the plant response to several abiotic stresses (Senaratna et al. 2000). SA mediates the oxidative burst that leads to cell death in the hypersensitive response, and acts as a signal for the development of the systemic acquired resistance (Shirasu et al. 1997). In this study, application of SA under drought stress enabled plants to alleviate drought stress-induced symptoms and to help plants grow normally (Table 1). Our observations were in agreement with the report of Gutiérrez et al. (1998), who reported a similar increase in the growth of shoots and roots of soybean plants in response to SA treatment. The improvement in the growth by SA treatment may be a result of the increase in the level of cell division within the apical meristem of seedling roots which caused an increase in plant growth (Sakhabutdinova et al. 2003). It was consistent with our results that SA treatment induced larger roots growth than shoots growth (Table 1). Thus, the ability of SA to increase plant dry mass, negating the adverse effect of water stress, may have significant implications in improving plant growth and overcoming the barrier arising from conditions of limited water availability.

In the present study, RWC declined in response to drought stress. However, in comparison with non SA-treated plants, SA-treated plants under drought stress exhibited a slower increase in RWC. This was supported by Ying et al. (2013), who found that exogenous SA treatment increased RWC in leaves of drought stressed Red bayberry plants. Plants had higher capacity of osmotic adjustment in terms of accumulating proline, which could maintain water absorption under drought stress (White et al. 2000). With SA treatment, more proline accumulation was observed in moderate water-stressed plants than that in severe water-stress plants (Fig. 1b). On the other hand, the RWC in SA-treated plants under moderate water stress did not significantly change in comparison to the control plants (Fig. 1a). Therefore, under moderate water stress, the improvement in RWC by exogenously application of SA may be a result of osmotic adjustment because of accumulation of compatible solutes like proline. Significantly lower RWC in severe water-stressed plants might be related to less accumulation of proline.

Stomatal closure is one of the earliest responses to drought stress, protecting plants from extensive water loss. In the present study it was observed that moderate water stress reduced Pn, Gs, Tr and Ci value. The decrease in photosynthetic rate mediated by moderate water stress was the consequence of the closure of stomata, thereby decreasing CO_2 supply as well as Ci (Ozkur et al. 2009) and resulting in a decrease in Tr. Compared with non SA-treated plants, plants treated with SA increased in Gs. This confirmed that SA application can reverse the stomatal closure of *T. grandis* seedlings under moderate water stress (Fig. 2a, b), and thereby increase in photosynthetic rate. However, non-stomatal limitations to Pn might also occur in leaves under stressful conditions. Our results showed that Ci was increased dramatically in severely stressed plants with reduced chlorophyll content (Table 2), Gs (Fig. 2a) and Tr (Fig. 2d), suggesting that the decrease in net photosynthetic rate (Pn) is primarily a consequence of the non-stomatal limitation. SA alleviates decrease in photosynthesis of grapevine leaves under heat stress through maintaining higher Rubisco activation (Wang et al. 2010). In this study, total chlorophyll content in *T. grandis* plants seedling decreased with the decreasing of the water availability. However, application of SA promoted the increase in chlorophyll contents in *T. grandis* plants seedling under water stress (Table 2). The conclusion was in accordance with the results found in jack pine seedling and wheat plants (Rajasekaran and Blum 1999; Loutfy et al. 2012). The increased chlorophyll content under SA treatment might be due to the ability of SA to increase the activity of some enzymes and thereby stimulate chlorophyll biosynthesis or reduce chlorophyll degradation. Thus, these results indicating that increased chlorophyll content in the plants treated with SA may be another reason for the increase in Pn.

Decrease in CO_2 assimilation under water deficit was due to closure of stomata (Ozkur et al. 2009); consequently, there was an accumulation of NADPH, which resulted in the formation of active oxygen species (AOS), causing damage to the membrane system and photosynthetic complexes (Asada 1999). Plants are able to protect their tissues from harmful effects of drought-accumulated AOS using enzymes such as SOD, CAT and APX (Verhagen et al. 2004). In the present study, SA had induced all antioxidant enzyme activities in *T. grandis* plants under drought condition (Fig. 3). There are many reports supporting the notion that application of SA can increase the activity of antioxidant enzymes such as CAT, POD and SOD (Hayat et al. 2008, 2010), which in turn protect plants against AOS generation and lipid peroxidation. For example, Wang and Li (2006) have previously reported that SA alleviates heat damage of grape plants by up-regulating the antioxidant system. In this work, a significant rise in the activity of CAT, POD and SOD in the SA-treated plants relative to non SA treatment revealed that SA exerted beneficial effects on drought tolerance of *T. grandis* by enhancing their antioxidative capacity (Fig. 3). MDA is an end product of lipid peroxidation in biomembranes and the MDA content usually reflects the level of lipid peroxidation that indirectly reflects the extent of membrane injury. In our study, we found that high level of MDA was correlated with high level of oxidative damage to lipid membranes ($r = 0.99$, $P < 0.05$). MDA content in SA-treated plants exposed to moderate water deficit was very close to that in control plants (Fig. 4). It indicates that antioxidant defense system induced by SA may protect plants under moderate water stress, while under moderate drought without SA application, an imbalance between production and scavenging systems of ROS may cause oxidative stress as could be judged by the accumulation of MDA and the increase in the relative electrolyte conductivity (Fig. 4). Moreover, we found that there is a strong negative correlation between chlorophyll and MDA contents ($r = 0.90$, $P < 0.05$). The reduction in chlorophyll due to osmotic stress has been ascribed to the strong damage of chloroplast membranes (Kaiser et al. 1981). Thus, we suggest that the accumulation of MDA severely damaged the chlorophyll molecules (Fig. 4).

In conclusion, exposure to water stress conditions resulted in decreased CO_2 assimilation and plant growth together with increased lipid peroxidation. SA could efficiently ameliorate the negative effects of moderate drought stress on the growth of *T. grandis* through up-regulating osmotic adjustment system, Gs and antioxidant enzymes activity to maintain good water status and to increase the biomass of plants. To our knowledge, this finding uncovers

a mechanism that has not been previously described in *T. grandis* seedlings under drought stress. The results of the study can also provide some references for the conservation and recovery of other species of Torreya.

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Conflict of interest The authors declare that they have no conflict of interest.

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