



Interactive effects of drought and shading on *Torreya grandis* seedlings: physiological and growth responses

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Abstract

Key message Moderate shading is optimal for the growth of *Torreya grandis* seedlings during drought.

Abstract Light and water are two important environmental factors affecting tree seedling traits and regulating seedling survival and growth, but the effects of light on seedlings under drought remain poorly understood. The purpose of the present study was to examine the interactive effects of light and drought on second-year *Torreya grandis* seedlings. A pot experiment was conducted with 240 seedlings using four shading treatments (0%, 50%, 75%, and 90% reduction of full sunlight, respectively) and two watering treatments (well watered = 75% of field capacity, drought treatment = 30% of field capacity). In drought-treated seedlings, photosynthesis was inhibited along with decreased contents of chlorophyll and high contents of reactive oxygen, and biomass accumulation was reduced, as compared to well-watered seedlings. Seedlings grown under moderate shade (50% and 75%) showed highest biomass and photosynthetic rate both in well-watered and drought treatments. Similarly, the differences between well-watered and drought-treated seedlings for stress metabolism parameters (i.e., contents of proline and soluble protein) were also lower under moderate shading, indicating drought stress was alleviated by the shading. The chlorophyll contents and the photosynthetic rate were higher in the seedlings under moderate shading than in those in full sunlight, contributing to a higher biomass in the former. These results suggest that moderate shading can effectively prevent stress caused to the *Torreya grandis* seedlings by excess light and can also alleviate the damage caused to them by drought.

Keywords Antioxidant enzymes · Biomass · Drought stress · Forest management · Photosynthesis · Shading

Introduction

Light and water are the main factors affecting plant traits, regulating plant survival and growth, and determining the distribution of plant species at the global scale (Quero et al. 2006). By the end of this century, the land surface temperature in China may rise by 1.3–5 °C and extreme climatic events will occur more frequently than at the present (Wu et al. 2016). It is foreseeable, then, that climate change will be an important factor affecting plant growth also in China. Among the changing environmental factors, the role of drought will be especially important in the future (Zelazowski et al. 2011; Zhou et al. 2003).

Drought is an important factor limiting plant performance (Lassouane et al. 2016), and the physiological and phenotypic responses to drought range from the molecular level to the whole-tree level (Allen et al. 2015; Ashraf and Harris 2013). Drought hampers the process of photosynthesis by reducing the ability of plants to supply water to

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the leaves (Ashraf and Harris 2013; Choat et al. 2012), leading to a subsequent increase of the concentrations of various stress metabolites (Krasensky and Jonak 2012). One key mechanism of plant adaptation to drought is to allocate biomass predominantly to roots and reduce allocation to leaf biomass (Tran et al. 2014; Weih et al. 2011). Acclimation to drought occurs via a wide range of physiological adjustments, such as up-regulation of water use efficiency, down-regulation of transpiration, increases in the contents of proline and soluble protein and enhanced activity of protective enzymes (Allen et al. 2015; Bandursha and Cieslak 2012).

In addition to water supply, light is another environmental factor critical to plant performance. Besides being the energy source of plants, light regulates several essential processes in plants, such as growth and development (Wit et al. 2016). The optimal intensity of light for plant growth is species specific. For instance, for shade-adapted species, excessive light has detrimental effects on plant growth due to the degradation of photosynthetic pigments and reduced photosynthesis (Escobar-Bravo et al. 2017).

However, the interactive effects of light and water on plants remain poorly understood, and there are contrasting hypotheses for this interaction. Some hypotheses state that shade alleviates, or at least does not aggravate, the effects of drought on plants (Quero et al. 2006). Accordingly, moderate shade contributes to the maintaining of stomatal conductance, so that photosynthesis is not slowed down due to the reduced supply of CO₂ under drought (Jaume et al. 2004; Quero et al. 2006). However, deep shade may aggravate the stress imposed by drought (Valladares and Pearcy 2002). This is caused by the trade-off of allocation, as under drought, plants tend to allocate biomass to the roots, whereas under shortage of light the allocation to the shoot is emphasized (Smith and Huston 1989; Valladares and Pearcy 2002). Contrary to the hypotheses of interactive effects of shade and drought discussed above, Sack (2004) posits that the impacts of shade and drought are independent of each other.

The coniferous tree species *Torreya grandis* is a primitive member of the yew family (Taxaceae). Due to its restricted distribution and great historical importance, it is considered as an ancient, precious and endangered species in China (Zhang et al. 2017b). *T. grandis* is one of the most important cultivated plants in China, as it has both ornamental, medicinal, and agricultural values (Chen et al. 2006; Cheng et al. 2007; Huang et al. 2001; Yu et al. 2016). *T. grandis* is mainly planted on hills and mountain slopes in southern China. It is often subjected to serious summer droughts, and artificial irrigation systems are not generally available in the plantations (Feng and Hong 2007; Zhou et al. 2003). Furthermore, *T. grandis* seedlings are shade adapted (Cheng et al. 2007), so that they are probably stressed by both drought and strong incoming solar radiation in the plantation. However,

no experimental results are available for the interaction of drought and light on the *T. grandis* seedling performance.

The objective of this study is to determine the responses of *T. grandis* seedlings to the increased frequency of events combining drought with strong incoming solar radiation under climate change. *T. grandis* seedlings were subjected to various shading and watering treatments, and subsequently the growth and physiological characteristics were investigated. We aimed to evaluate (1) how drought and light affect the physiological and growth traits of the seedlings, (2) whether shading alleviates the stress caused by drought to the seedlings, and (3) how the responses of the physiological traits are related to the responses of growth under the combination of drought and shading. Examining these phenomena will provide management guidance for the regeneration of *T. grandis* under climate change.

Materials and methods

Experimental site and plant material

This experiment was carried out in a greenhouse of Zhejiang Agriculture and Forestry University (30°23'N, 119°72'E), Zhejiang Province, China. Second-year uniform and healthy seedlings of *T. grandis* were used as experimental material. The seedlings were selected from a commercial nursery located in Zhuji (29°48'N, 120°28'E), Zhejiang Province, where they had been raised using standard management practices. Seedlings were grown in pots (13 × 17 cm) filled with pine bark, peat and local soil (4:4:2, v/v/v). Seedlings were irrigated with sprinklers to keep the growth medium well watered. Weeds were removed by hand as needed. In natural conditions, bud burst takes usually place in *T. grandis* seedlings from late April to early May. During their second year, the height growth of the seedlings is typically limited (Tang et al. 2015), whereas branches become profuse (Dai et al. 2005).

Starting on 10 April 2015, 300 seedlings were transplanted into plastic pots of 22 cm diameter and 25 cm height. The height and stem diameter of the transplanted seedlings were 50.1 ± 5.1 cm and 7.2 ± 1.0 mm, respectively. One seedling per pot was transplanted into 1.55 kg of artificial growing medium consisting mainly of pine bark, peat and soil (4:4:2, v/v/v). Soil belonging to the Hapludult type in soil taxonomy was collected from the local field. The growing medium contained 40 kg m⁻³ organic fertilizer consisting of 714 mg kg⁻¹, 86.1 mg kg⁻¹, and 512 mg kg⁻¹ of available N, P and K, respectively. The pH of the substrate varied between 6.3 and 6.5. The growing medium was considered to be sufficiently fertile, so no fertilizer was applied. After transplanting, pots were maintained well watered at 75% water-holding capacity by weight. During the experiment

from May to September, the night temperature in the greenhouse was approximately 18 °C and the day temperature varied between 26 and 32 °C. The relative humidity of air varied between 50 and 80%. In the greenhouse, bud burst took place around 2 May. After growing the seedlings for 1 month in the greenhouse, the height and stem diameter of the seedlings were measured before starting the experiments on 20 May 2015. The height was measured by a ruler (accuracy 0.1 cm) and the diameter by a caliper (accuracy 0.02 mm).

Experimental design

A total of 240 seedlings were selected for a 4×2 factorial experiment consisting of four levels of shading and two levels of watering. The experiment was set up as a completely randomized design with three replicates per treatment and ten plants per replicate. The shading treatments were identified by the percentual reduction of light intensity measured right above the seedlings, as compared with the light intensity measured right above the unshaded control seedlings: 0% (control, full sunlight), 50%, 75%, and 90% reduction. The shading was set using different number of layers of neutral black curtains not affecting the spectral composition of light. In each shading treatment site, photosynthetically active radiation (PAR) was measured every 2 h during sunny day with a Digital Lux Meter (TES-1339R, Taiwan, China). The PAR was measured at five positions (east, west, north, south and middle) in each shading treatment location, and the results of these measurements confirmed that shading with the layers of the black curtain created different levels of light intensity as designed (Table S1). In the two watering treatments, the water content of the growing medium was held at 75% (well watered, WW) and 30% (drought treatment, DT) of field capacity, respectively. Each pot was weighted every 1–3 days depending on the greenhouse conditions and watered with distilled water to maintain the designed water content of the growing medium (Valliere and Allen 2016). The experimental treatments were conducted until late September, when the measurements of the growth and physiological parameters were carried out (see below for exact dates).

Measurements of growth, biomass, and water content

The height and stem diameter of all seedlings were measured once more on 20 September 2015 and the growth increments of both height and diameter during the experiment were subsequently calculated. For biomass measurements, nine seedlings were sampled from each treatment on 20 September 2015. The root, stem and leaves of each seedling were separated with scissors after lifting the seedling out

from the pot and washing out the growth medium remaining attached to the roots. After that, the different compartments were kept in an oven, first half an hour in 105 °C and then in 80 °C until a constant weight was obtained for each compartment. The leaves were oven-dried for 24 h and the stems and roots for 72 h.

A piece of 2 mm length was cut off from both ends of the leaves (needles), and the rest of the leaf was used for RWC (relative water content) measurement. About 0.5 g of leaves was weighed immediately after sampling (fresh weight, W_f), then once more after rehydrating them for 24 h in darkness (saturated weight, W_s) and finally after drying them in oven at 85 °C for 24 h to a constant mass (dry weight, W_d). The RWC was then calculated using the formula: $RWC (\%) = (W_f - W_d) / (W_s - W_d) \times 100$ (Shen et al. 2014).

Measurements of gas exchange and chlorophyll fluorescence

On 26 September 2015, five seedlings per treatment were randomly sampled for measurements of gas exchange. Fully developed branches were randomly selected from each seedling for the measurements. The measurements were carried out with a portable photosynthesis measuring instrument (LI-6400, LiCor, Inc. Lincoln, NE, USA) equipped with a 6400-05 conifer chamber and 6400-18 RGB artificial light source. The measurements lasted 5 days and were conducted on sunny days from 8:30 to 11:00 AM at an air concentration of 21% O₂, 400 μmol mol⁻¹ CO₂, 50% relative humidity, and air temperature of 28–30 °C. In the successive measurements, the PAR was set automatically at 1500, 1200, 1000, 800, 600, 400, 200, 150, 100, 50 and 0 μmol m⁻² s⁻¹. Before measurements, leaves were kept in 1000 μmol m⁻² s⁻¹ PAR for photoinduction. After measuring the gas exchange, the measured branch was detached and its total leaf area was measured by counting the number of 1 mm × 1 mm squares on its contour. The quantum yield efficiency (AQE, μmol m⁻² s⁻¹), the light compensation point (LCP, μmol m⁻² s⁻¹), the light saturation point (LSP, μmol m⁻² s⁻¹) and the maximum photosynthetic rate (P_{max}) were determined using an AQ response curve analysis software (Version 1.0, LI-COR, 2/2008; Xu et al. 2016).

Chlorophyll fluorescence measurements were made on 26 September 2015 with the same seedlings that were sampled for the gas exchange measurements. From each seedling, three young and fully developed branches were randomly sampled for the measurements carried out with the PAM2500 Fluorescence Meter (Walz Effeltrich Germany). The measurements were carried out after 30-min dark adaptation (Tang et al. 2015). The minimum fluorescence (F_o) was determined in approximately 0.5 μmol photon m⁻² s⁻¹, and the maximum fluorescence (F_m) under a 0.8-s saturating

pulse of 10,000 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$. The F_v/F_m value was calculated as $(F_m - F_o)/F_m$ (Maxwell and Johnson 2000).

Measurements of photosynthetic pigments

The chlorophyll contents were measured with the alcohol extraction method (Arnon 1949). Three branches per seedling and about eight youngest healthy and fully developed leaves per branch were randomly sampled from the same seedlings that were sampled for the gas exchange measurements. The chlorophylls were extracted by incubating a sample of about 0.1 g of finely cut and well-mixed leaf mass in 8 ml 95% ethanol at 4 °C for 24 h in darkness and by shaking three or four times until the leaves were blanched. The absorbance of the extract was measured with a spectrophotometer Shimadzu UV-2550 (Kyoto, Japan) at 470 nm, 649 nm and 665 nm after centrifugation of the mixture on standing. Chlorophyll concentrations were calculated by the standard method of Lichtenthaler (Hartmut 1987) and expressed in mg/g fresh weight (FW).

Measurement of stress metabolites and enzyme activities

Six seedlings per treatment were randomly sampled for physiological laboratory measurements. In each seedling, 10 branches and about 20 youngest healthy and fully developed leaves per branch were selected. After sampling, the leaves were quickly frozen and stored in a nitrogen canister. The leaves were then transferred into laboratory freezer where they were stored at -80 °C. On 29 September 2015, the leaves were taken out from the freezer and cut with scissors into 2-mm pieces. A leaf sample of 0.5 g was put in a pre-cooled mortar, where 2 ml pre-cooled phosphate buffer (pH 7.8, 0.05 mmol l^{-1}) was added with a pipette. The ice bath was ground into a slurry, the slurry was poured into a 10-ml centrifuge tube and washed twice with 3 ml of the phosphate buffer. Then 8 ml of the slurry was transferred to a centrifuge tube and centrifuged at 9000 rpm for 20 min at 4 °C. The supernatant with the extracted enzymes was achieved and was carefully transferred to a 10-ml centrifuge tube and stored in the refrigerator at 0–4 °C. Subsequently, the enzyme activities were measured using the NBT method for superoxide dismutase, SOD (Maxwell and Johnson 2000), the guaiacol method for peroxidase, POD (Dixo and Paiva 1995), and the assay method based on UV absorption for catalase, CAT (Chen et al. 1993).

The rate of $\text{O}_2^{\cdot-}$ production was determined using the hydroxylamine oxidation method (Wang and Luo 1990). The H_2O_2 content was determined using the method of Patterson and Mackae (1984). Proline content was determined using the method described by Bates et al. (1973), with the slight modifications described by Shen et al. (2014). Soluble

protein content was measured according to the Bradford's method using Coomassie brilliant blue (Bradford 1976). A 0.5-g frozen sample was ground with 5 ml sodium phosphate buffer (50 mmol/L, pH 7.8); then the mixture was transferred into a 10-ml centrifuge tube and centrifugated at 10,000 rpm for 15 min. After centrifugation, a 0.1 ml extract from each centrifuge tube was mixed with 0.9 ml of distilled water and 5 ml Coomassie brilliant blue G-250. The control group was prepared similarly but instead of the extract, distilled water was used. The absorbance was measured at 520 nm after 2 min incubation. The soluble protein content was determined from a standard curve and calculated on a fresh weight basis as follows.

Soluble protein content (mg g^{-1}) = $(C \times V_1) / (W_f \times V_s \times 10^3)$, where C is the soluble protein content given by the standard curve (μg), V_1 the total volume of the extract (ml), V_s the volume of the extracting liquid before adding the sample (ml) and W_f the fresh weight of the sample (g).

Statistical analyses

To evaluate effects of watering and shading treatments on the growth and physiological traits, data were subjected to analysis of variance (ANOVA) and mean separation was done by Fisher's least significance difference (LSD) at $P \leq 0.05$. The data are presented as the mean \pm SD. The interactive effects of watering and shading on growth and physiological traits were analyzed by a two-way ANOVA. Before ANOVA, data were checked for normality and homogeneity of variances. When necessary, the data were log-transformed to account for deviations from these assumptions. All statistical analyses were carried out with the SPSS software (Version 16.0, SPSS Inc., Chicago, USA).

Results

Growth and biomass allocation

Drought affected significantly the growth traits of the seedlings (Table S2, Fig. 1). Compared to seedlings in well-watered treatments, drought treatment decreased the total biomass, and increased the root:shoot ratio. Light, interacting with water, significantly affected the height and stem diameter increment (Table S2), but the total biomass was not affected by their interaction (Fig. 1a). In both well-watered and drought treatments, shading increased the total biomass. Especially, moderate shading (50% and 75%) resulted in higher total biomass compared to extreme shading (90%). The interaction of light and water on biomass allocation was significant (Fig. 1b). In full sunlight and under 50% shading, drought caused more allocation to roots, but under 75% and

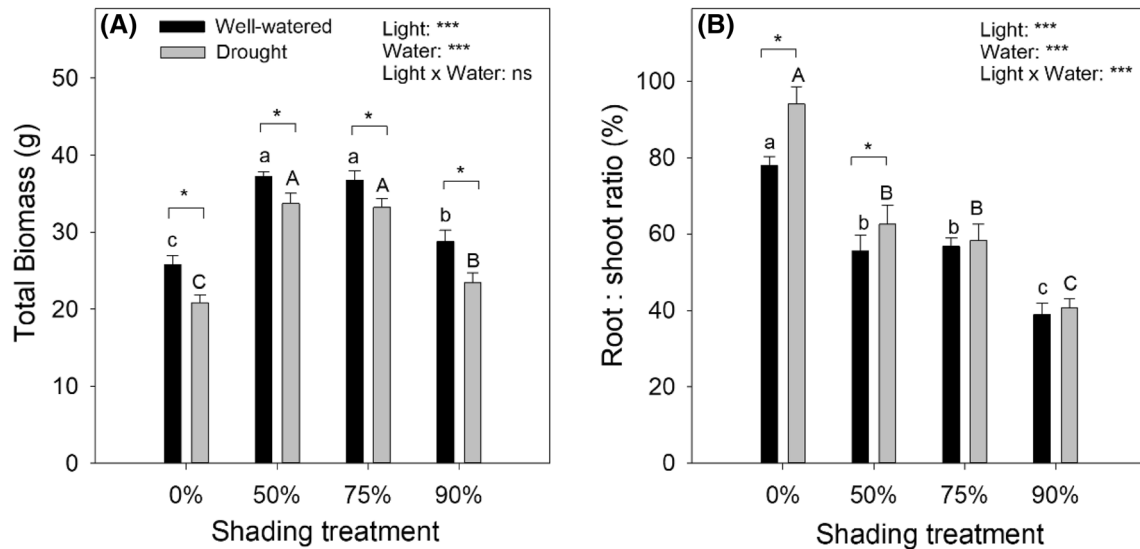


Fig. 1 The total biomass (a) and root:shoot ratio (b) of *Torreya grandis* seedlings under various shading and watering treatments. Well watered=75% of field capacity, drought treatment=30% of field capacity. The shading treatment is identified by the percentage of shading (Table S1). Bars with different letters indicate statistically significant differences among shading treatments in well-watered seedlings (lower case letters) and drought-treated seedlings (upper

case letters). Asterisks indicate statistically significant differences between the two watering treatments at the same shading level. All statistical significances were determined at $P \leq 0.05$. A summary of the results of a two-way ANOVA addressing the effects of water and light is seen in the upper right-hand corner of each panel (* $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, non-significant)

90% shading, drought did not change the root:shoot ratio, as compared with the seedlings in well-watered treatments.

Gas exchange, photosynthetic pigments and chlorophyll fluorescence

Numerous photosynthetic traits were significantly affected by light and water, and their interaction was also often significant (Table S3). The maximum photosynthetic rate (P_{max}) was significantly affected by light and water, but their interaction was not significant (Fig. 2a). In each shading treatment, drought decreased P_{max} . In both well-watered and drought treatments, shading increased P_{max} , as compared with seedlings in full sunlight treatments.

The contents of photosynthetic pigments were significantly affected by shading and drought (Table S4, Fig. 2b). Compared to well-watered conditions, drought decreased significantly the contents of all of the photosynthetic pigments under 0% and 90% shade but had no significant effect on them at the moderate levels of shading (50% and 75%) (Fig. 2b).

The maximal quantum yield of photosystem II (F_v/F_m) was significantly affected by light and water, and their interaction was also significant. Compared to full sunlight treatments, shading increased significantly F_v/F_m (Fig. 3). At each level of shading, drought decreased F_v/F_m , except that it did not affect F_v/F_m under 75% shading.

Relative water content and contents of proline and soluble protein

The effects of water and light, and their interaction, on RWC were all significant (Fig. 4a). Compared to well-watered conditions, drought significantly decreased RWC in all shading treatments. Furthermore, in the well-watered seedlings, there were no statistically significant differences in RWC among the treatments with 50–90% shading, whereas in the drought-treated seedlings, the RWC was significantly highest in the 75% shading. The contents of both proline and soluble protein were significantly affected by light and water, and their interaction was also significant. Their responses to drought and shading showed similar patterns (Fig. 5b, c). Under 0% and 90% shading, drought increased significantly the contents of proline and soluble protein, but under 50% and 75% shading, drought did not have any effect on them, as compared with the proline and soluble protein content in well-watered seedlings.

Stress metabolites and enzyme activities

Regardless of the watering treatment, the production rate of O_2^- , the content of H_2O_2 , and the activities of SOD, POD, and CAT were higher in the treatments with no (0%) or extreme (90%) shading than in the treatments with moderate shading (50%, 75%) (Figs. 5, 6). Compared with the well-watered seedlings, drought significantly increased the production rate of

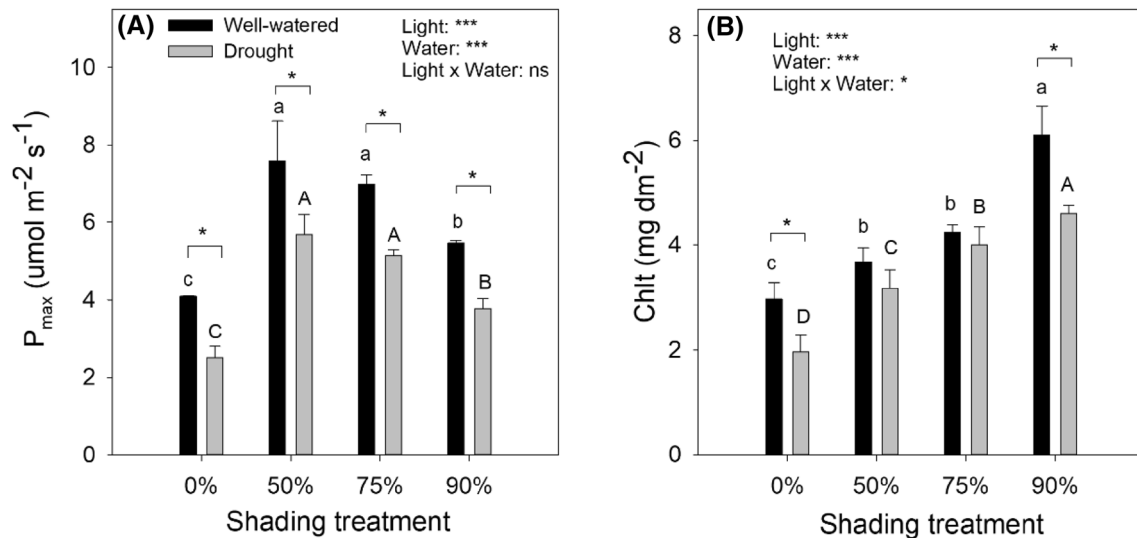


Fig. 2 The maximal net photosynthetic rate (P_{max}) (a) and the total chlorophyll content (b) of *Torreya grandis* seedlings under various shading and watering treatments. Well watered=75% of field capacity, drought treatment=30% of field capacity. The shading treatment is identified by the percentage of shading (Table S1). Bars with different letters indicate statistically significant differences among shading treatments in well-watered seedlings (lower case letters) and drought-

treated seedlings (upper case letters). Asterisks indicate statistically significant differences between the two watering treatments at the same shading level. All statistical significances were determined at $P \leq 0.05$. A summary of the results of a two-way ANOVA addressing the effects of water and light is seen in the upper right-hand (A) and left-hand (B) corner of panels ($*P \leq 0.05$; $**P < 0.01$; $***P < 0.001$; ns, non-significant)

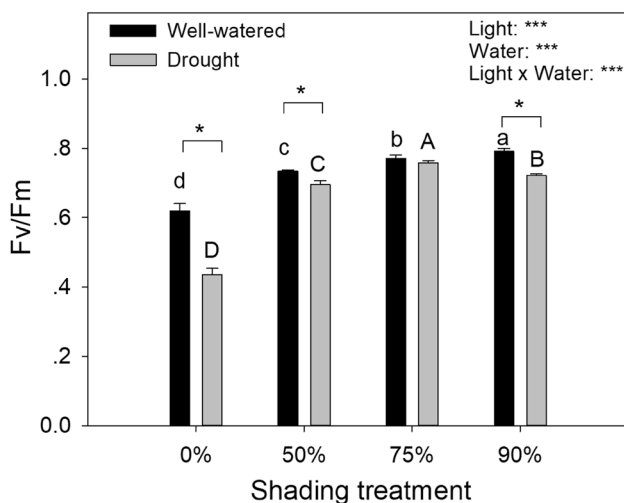


Fig. 3 The maximal photochemical efficiency (F_v/F_m) of *Torreya grandis* seedlings under various shading and watering treatments. Well watered=75% of field capacity, drought treatment=30% of field capacity. The shading treatment is identified by the percentage of shading (Table S1). Bars with different letters indicate statistically significant differences among shading treatments in well-watered seedlings (lower case letters) and drought-treated seedlings (upper case letters). Asterisks indicate statistically significant differences between the two watering treatments at the same shading level. All statistical significances were determined at $P \leq 0.05$. A summary of the results of a two-way ANOVA addressing the effects of water and light is seen in the upper right-hand corner of the panel ($*P \leq 0.05$; $**P < 0.01$; $***P < 0.001$; ns, non-significant)

O_2^- (Fig. 5a). The content of H_2O_2 was increased significantly by drought treatment under 0% and 90% shading, but drought treatment did not affect it significantly under 50% and 75% shading (Fig. 5b). Similar patterns were found for the activities of SOD (Fig. 6a) and CAT (Fig. 6c). The effects of drought treatment for the activity of POD were otherwise similar but its activity was increased significantly by drought treatment also in the 50% shading treatment (Fig. 6b).

Discussion

In the present study, both drought and extremely high levels of light (full sunlight) limited growth of *T. grandis* seedlings. Although light and water affected independently the photosynthetic rate and biomass production, numerous significant interactions of these two factors were found in other physiological traits. Effects of drought were alleviated in moderate shade because, in most cases, the differences between well-watered and drought-treated seedlings in stress metabolism parameters, such as contents of proline and soluble protein, were smaller under moderate shading.

Effects of drought and light on *Torreya grandis* seedlings

As a result of decreased photosynthetic rate both drought and full sunlight decreased in the present study the total biomass accumulation of *T. grandis* seedlings (Fig. 1a). The

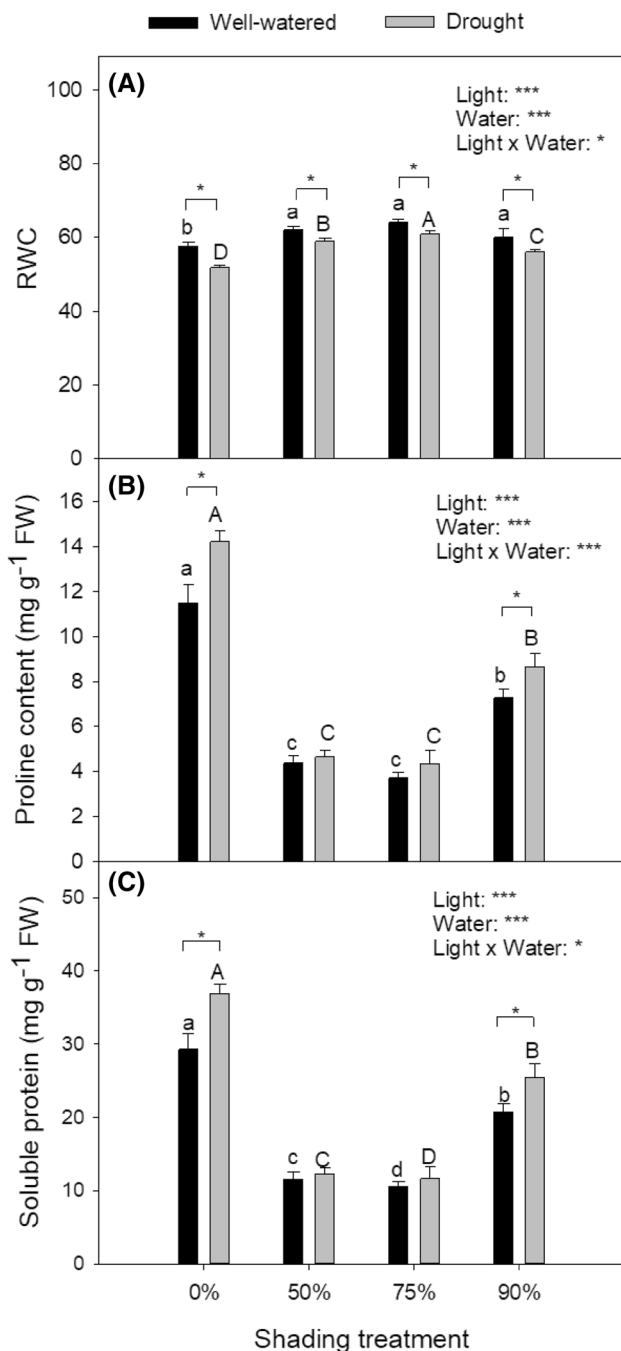


Fig. 4 Relative water content (RWC) (a), content of proline (b), and content of soluble protein (c) of *Torreya grandis* seedlings under various shading and watering treatments. Well watered=75% of field capacity, drought treatment=30% of field capacity. The shading treatment is identified by the percentage of shading (Table S1). Bars with different letters indicate statistically significant differences among shading treatments in well-watered seedlings (lower case letters) and drought-treated seedlings (upper case letters). Asterisks indicate statistically significant differences between the two watering treatments at the same shading level. All statistical significances were determined at $P \leq 0.05$. A summary of the results of a two-way ANOVA addressing the effects of water and light is seen in the upper right-hand corner of each panel (* $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, non-significant)

decreased photosynthesis was associated with decreases of total chlorophyll content and chlorophyll fluorescence (Fig. 2), indicating damage to the structure and functioning of the chloroplast membrane (Anna and Alicja 2001; Li et al. 2004; Ashraf 2003; Sun and Li 2017) and a decreased quantum efficiency of photosystem PSII photochemistry (Murchie and Lawson 2013; Paulo et al. 2012; Zhang et al. 2017a). Also, both drought and full sunlight led to decreased relative water content (RWC) (Fig. 4a), indicating the loss of cellular water. The loss is detrimental to the seedlings because the cellular water is needed for maintaining the integrity of the chloroplast structure, functioning of PSII, and finally the photosynthetic production of the seedlings (Colom and Vazza 2003).

Both drought and full sunlight may cause lipid peroxidation and membrane damage (Ma et al. 2015), as indicated by the increased production rate of $O_2^{\cdot-}$ and increased H_2O_2 content observed in the present study (Fig. 5). This is consistent with the enhanced activities of antioxidant enzymes (i.e., SOD, POD and CAT) also observed under extreme light and drought conditions in the present study (Fig. 6), indicating an enhancement of the scavenging ability of reactive oxygen species (ROS) (Shen et al. 2014). Furthermore, values of other stress metabolism parameters, such as contents of proline and soluble protein (Fig. 4b, c), were also increased by drought or full sunlight, which is a sign of plant acclimation to drought or full sunlight by maintaining cell turgor and increasing water-absorbing and water-holding capacity of the cells (Turner 1975).

T. grandis is a shade-adapted species (Cheng et al. 2007). Accordingly, Tang et al. (2015) suggested moderate shading is optimal for *T. grandis* growth under well-watered conditions. In the present study, we found that shading is beneficial to the growth of *T. grandis* seedlings under both well-watered and drought conditions. Shading increased total chlorophyll content and enhanced photosynthesis, and in this way increased total biomass, as compared with treatments under full sunlight conditions (Figs. 1, 2). Furthermore, shading was beneficial for the photosynthetic apparatus of the *T. grandis* seedlings (Anna and Alicja 2001; Li et al. 2004), as indicated by the increased F_v/F_m under shading treatments relative to full sunlight treatment (Fig. 3). Shading treatments efficiently increased the chlorophyll content of leaves of the *T. grandis* seedlings (Fig. 2), indicating an accelerated synthesis of chlorophylls under shading. Similar results about the facilitation of photosynthesis and growth by shading have been earlier found for *Leymus chinensis*, a grass species (Yang et al. 2017), and *Myrica rubra*, a fruit tree species (Zeng et al. 2017).

However, the photosynthetic rate was lower under deep shading (90%) than under moderate shading (50% and 75%) (Fig. 2a), indicating that insufficient light limited photosynthesis under the deep shading. Consequently, the total

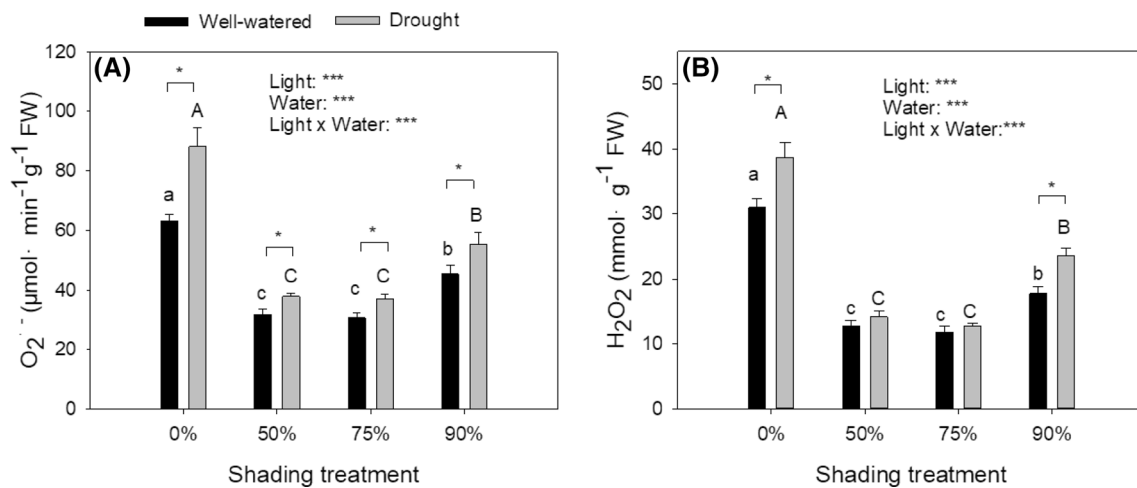


Fig. 5 The O₂⁻ production rate (a) and the H₂O₂ content (b) of *Torreyia grandis* seedlings under various shading and watering treatments. Well watered = 75% of field capacity, drought treatment = 30% of field capacity. The shading treatment is identified by the percentage of shading (Table S1). Bars with different letters indicate statistically significant differences among shading treatments in well-watered seedlings (lower case letters) and drought-treated seedlings

(upper case letters). Asterisks indicate statistically significant differences between the two watering treatments at the same shading level. All statistical significances were determined at $P \leq 0.05$. A summary of the results of a two-way ANOVA addressing the effects of water and light is seen in the upper middle of each panel (* $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, non-significant)

biomass was lower under deep shade relative to that under moderate shade (Fig. 1a). To reduce damage caused by environmental stress, plants have a variety of mechanisms, such as increasing the activities of antioxidant enzymes to resist the increasing content of reactive oxygen species ROS (Sarvajeet and Narendra 2010) and increasing the content of proline and soluble protein (Matysik et al. 2002). In the present study, the soluble protein content and the proline content were much higher under full sunlight and deep shade than under moderate shade (Fig. 4b, c). Similar results were found for the activities of the antioxidant enzymes. These results indicate that besides extremely high levels of light, also deep shade causes stress to the *T. grandis* seedlings, where the scavenging ability was enhanced to remove the reactive oxygen species through their active oxygen scavenging system.

The interactive effects of light and drought on *Torreyia grandis* seedlings

The interactive effects of light and water on plant performance remain poorly understood, as both alleviation and aggravation of the effects of drought on plants by shading have been reported (Valladares and Pearcy 2002; Quero et al. 2006; Escobar-Bravo et al. 2017). In our study, the interactive effects of light and water on the photosynthetic rate and biomass production in *T. grandis* seedlings were not significant. This is in line with the hypothesis posited by Sack (2004) stating that the effects of shade and drought on plants are independent of each other. Accordingly, we found

that both drought and full sunlight limited the growth of *T. grandis* seedlings by reduced photosynthetic rate. Compared to full sunlight, shading increased significantly the photosynthetic rate and biomass production both in well-watered and drought-treated seedlings (Fig. 2a).

Although light and water affected independently the photosynthetic rate and biomass production in the *T. grandis* seedlings, numerous significant interactions were found in the present study in other morphological and physiological traits. These included biomass allocation (Fig. 1b), contents of photosynthetic pigments (Fig. 2b), chlorophyll fluorescence (Fig. 3), contents of stress metabolites and antioxidant enzyme activities (Figs. 5, 6).

In general, plants acclimate to drought by increasing biomass allocation to roots under drought stress (Karina et al. 2012; Tran et al. 2014; Weih et al. 2011). We found that drought increased allocation to roots in *T. grandis* seedlings under full sunlight (0% shading) and under 50% shading, but not under 75% or 90% shading (Fig. 1b). These results suggest that a stronger shading (75% or 90%) prevented the changes in biomass allocation caused by drought observed with no or weak shading. This is consistent with the earlier findings with *Pinus pinea* seedlings (Pardos and Calama 2017), but opposite to those with European beech (*Fagus sylvatica* (L.)) (Schall et al. 2012). Although biomass allocation of *T. grandis* seedlings was not affected by drought either under moderate 75% or deep 90% shading, biomass production and photosynthetic rate were much higher under 75% than under 90% shading (Figs. 1a, 2a), suggesting that extreme shading limited the growth of the *T. grandis*

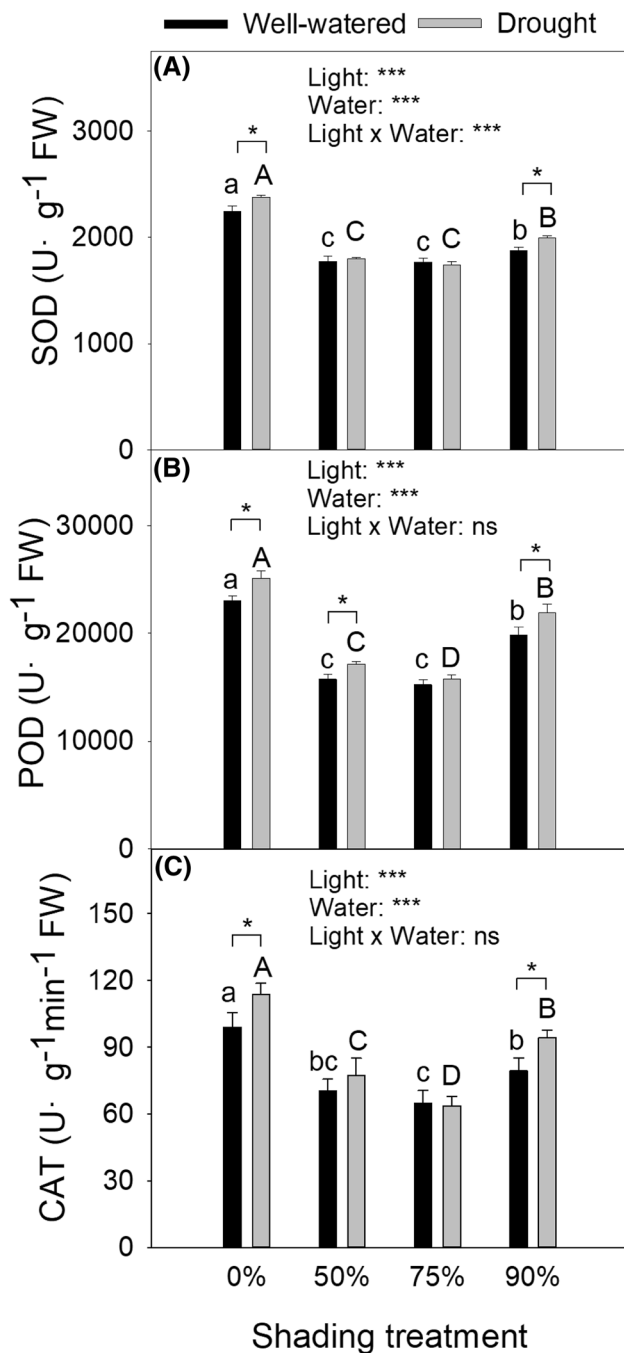


Fig. 6 Activities of superoxide dismutase, SOD, (a); peroxidase, POD, (b); and catalase, CAT (c) in *Torreyia grandis* seedlings under various shading and watering treatments. Well watered=75% of field capacity, drought treatment=30% of field capacity. The shading treatment is identified by the percentage of shading (Table S1). Bars with different letters indicate statistically significant differences among shading treatments in well-watered seedlings (lower case letters) and drought-treated seedlings (upper case letters). Asterisks indicate statistically significant differences between the two watering treatments at the same shading level. All statistical significances were determined at $P \leq 0.05$. A summary of the results of a two-way ANOVA addressing the effects of water and light is seen in the upper middle of each panel (* $P \leq 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns, non-significant)

seedlings. A possible explanation is that under extremely deep shade, plant performance is less influenced by the lack of other resources, such as water (Canham et al. 1996; Perrin and Mitchell 2013). Under the combination of deep shading and drought condition, light may be a more limiting factor for the growth of *T. grandis* seedlings, as compared to water. Accordingly, under deep shading, *T. grandis* seedlings allocated biomass primarily to the aerial parts, to facilitate capturing of the incoming restricted solar energy.

In the case of the moderate 75% shading, we found additional evidence to the notion that drought stress was alleviated by the shading. The differences between well-watered and drought-treated seedlings in many photosynthetic and stress metabolism parameters were smaller under moderate 75% shading than under full sunlight, or deep shade. For instance, the maximal quantum yield of photosystem II (F_v/F_m) was not affected by drought under 75% shade (Fig. 3), indicating that 75% shade can protect seedlings from damage to the photosynthetic apparatus caused by drought (Anna and Alicja 2001; Paulo et al. 2012). Furthermore, 75% shade alleviated lipid peroxidation and membrane damage caused by excessive oxygen anion production induced by drought (Ma et al. 2015), as indicated by a low level of H_2O_2 for the treatment combining 75% shade with drought (Fig. 5b). Other stress metabolism parameters, such as contents of proline and soluble protein, and activities of SOD, POD and CAT, were not influenced by drought under 75% shade (Figs. 4b, c, 6). These results indicated that, even though photosynthetic rate and biomass accumulation were decreased by drought also under 75% shading, the shading nevertheless alleviated the detrimental effects of drought on the physiological processes of the *T. grandis* seedlings.

Conclusions

This study provides a novel comprehensive evaluation of multiple growth and physiological responses to drought and shading in *Torreyia grandis* seedlings. This information is needed for determining the optimal management practices for growing the seedlings under the increased frequency of events combining drought with strong incoming solar radiation under climate change. Our results suggested that both drought and full sunlight are limiting factors for growth of *T. grandis* seedling. In both well-watered and drought-treated seedlings, moderate shading (50–75%) resulted in increased photosynthetic rate and increased biomass accumulation, as compared with seedlings exposed to full sunlight, or extreme deep shading (90%). Although water and light independently impacted the photosynthetic rate and biomass accumulation, for numerous morphological and physiological traits, their interactions were significant. Based on our results, 75% shading is optimal for alleviating the detrimental effects of

drought by maintaining the chloroplast structure and reducing the accumulation of reactive oxygen species, facilitating in this way maintenance of high photosynthetic rate and biomass accumulation.

Author contribution statement JW conceived and designed the experiment. JL, YH and YS performed experiments. RZ and JL analyzed the data. RZ, JL and HH wrote the manuscript. JL and RZ contributed equally.

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Compliance with ethical standards

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

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