



Physiological and microstructural responses of two *Rhododendron* cultivars to high temperature and low light

Yuan Li¹ · Wen Liang¹ · Bing Zhao¹

Received: 15 June 2019 / Revised: 8 January 2020 / Accepted: 26 January 2020 / Published online: 21 April 2020
© Korean Society for Horticultural Science 2020

Abstract

Rhododendrons (*Rhododendron*) are ornamental plants that exhibit poor thermotolerance. To provide a theoretical basis for the production and application of *Rhododendron* in summer, effects of high temperature and low light stress on the growth, physiological characteristics (including photosynthesis), and leaf structure of *Rhododendron* were explored. Two *Rhododendron* cultivars, *Rh.* ‘Fen Zhenzhu’ and *Rh.* ‘Zhuangyuan Hong’, were selected. *Rhododendron* plants were subjected to stress in 6-day intervals in an artificial climate box with 37.5, 75, 112.5, and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux at 38 °C. The leaf anatomical parameters and physiological indexes, including gas exchange parameters, relative water content, relative conductivity, chlorophyll and osmotic substance contents, malondialdehyde and hydrogen peroxide levels, and enzyme activity, of *Rhododendron* under high temperature and different low light levels were measured. The results showed that the combined stressors of high temperature and low light caused damage to leaves, such as loss of water, cell membrane damage, decreased net photosynthetic rate, and changes in leaf structure. Additionally, with the decreased light intensity caused more damage, and different damage mechanisms were found in different cultivars and treatments.

Keywords Anatomic structure · Appearance · Light intensity · Photosynthesis · Physiological indexes

1 Introduction

Plants are frequently subjected to various abiotic stressors such as unsuitable temperature, water, light, salinity, and heavy metals in natural or artificial conditions. In most cases, plants suffer from combined stressors at the same time, for example, high light and high temperature, which negatively affects plant growth and development (Vasseur et al. 2011; Soto et al. 2014; Lu et al. 2017; Wu et al. 2017). Lu et al. (2017) demonstrated that high temperature and high light caused serious growth and development obstacles, altering mesophyll cells’ gas diffusion and affecting photosynthetic carbon assimilation, multiple sites in photosystem II and photosystem I, reactive oxygen species (ROS) accumulation. These results suggest the significance of avoiding the removal of plants from high light in summer. *Rhododendron*

is one of the top 10 traditional flowers in China and is also a famous ornamental plant in the world. Owing to the colorful appearance and long blooming time of its flowers, *Rhododendron* has long been used in landscape plantings in Europe and North America (Kondratovics and Kondratovics 2017). *Rhododendron* is an acid soil plant that grows poorly in alkaline soil. However, most of the current cultivars of *Rhododendron* are sensitive to heat and light because of genetic factors (Vainola and Junttila 1998; Christiaens et al. 2014). *Rhododendron* prefers a cool, humid, and ventilated semishady environment, not tolerating either extreme heat or severe cold. It needs shady conditions or equipment when it is cultivated. The optimum growth temperature is 18–25 °C. When the temperature is over 30 °C, it grows slowly. *Rhododendron* is sensitive to water and does not withstand floods and droughts. Research has shown that the optimum light intensity for *Rhododendron* is 12,000 to 30,000 lx and no less than 3000 lx (Tao et al. 2015). *Rhododendron* is suitable for growth under scattered light with low light intensity in summer. If the light is too strong, the young leaves will be easily burned, and new leaves and old leaves are burnt, which will lead to plant death in serious cases.

Communicated by So-Young Park, Ph.D.

✉ Bing Zhao
bingzhao@nwsuaf.edu.cn

¹ College of Landscape Architecture and Arts, Northwest A&F University, Yangling 712100, Shaanxi, China

The effect of high temperature on *Rhododendron* is mainly reflected in its growth, leaf structure, physiological metabolism, and photosynthetic system. Due to high temperatures and high light in summer, the ornamental values of *Rhododendron* decrease dramatically because of the leaves and flowers, which are significantly damaged. Also, processes including extension of leaves, chlorophyll synthesis, photosynthesis, enzymatic activities, and accumulation of osmotic adjustment solutes are heavily affected (Gu et al. 2016; Zhou et al. 2017; Ihuyen et al. 2018). High temperature has become a major environmental factor limiting plant distribution, growth, and production (Grover et al. 2013). Therefore, it is important to study the heat tolerance of garden plants and how to improve their heat tolerance. At present, the methods to increase the heat tolerance of *Rhododendron* are not only to find heat-resistant resources and select and cultivate heat-resistant cultivars, but also to alleviate the damage caused by high temperature through cultivation and management under high temperature stress, such as improving light, adjusting air humidity, changing soil moisture conditions, and adequate fertilization. Hence, it is necessary to alleviate damage caused by combined stressors by planting *Rhododendron* under different shade or low light conditions in summer, like architectural shadows and tree shades, as this can help prevent the aforementioned damage from occurring (Shen et al. 2017; Zhou et al. 2017). This also offers the possibility of achieving the maximum utilization of light by planting other shrubs or trees, like a typical conifer species, with *Rhododendron* (Zhang et al. 2015; Reinhardt and Smith 2016).

Damage caused by temperature or light intensity can be evaluated through morpho-anatomical changes of leaves or through physiological processes like photosynthesis and oxidative damage (Bita and Tom 2013; Chen et al. 2014; Zhou et al. 2017). However, under high temperatures, what extent of shading or low light can limit the growth appearance of *Rhododendron*? Will lower light result in fewer negative effects? Two *Rhododendron* cultivars, *Rh.* ‘Fen Zhenzhu’ and *Rh.* ‘Zhuangyuan Hong’, were selected. *Rh.* ‘Fen Zhenzhu’ has strong heat resistance, and *Rh.* ‘Zhuangyuan Hong’ has weak heat resistance (Shen and Zhao 2018). In this study, the effects of different low light intensities on photosynthesis, cell membrane thermostability, osmoregulation substances, ROS scavenging ability, and anatomical morphology of two *Rhododendron* cultivars under high temperature were studied to explore whether lower light resulted in mitigated negative effects. By determining more suitable low light levels when *Rhododendron* is subjected to heat stress, we found a new way to effectively improve the high temperature resistance of *Rhododendron*, to alleviate the high temperature heat damage during cultivation, and to reduce the energy consumption under artificially controlled environments. In addition, we further the understanding of

the production and use of *Rhododendron* in summer, thereby providing a reference for improving its planning and planting pattern.

2 Methods and materials

2.1 Plant materials and treatments

In November 2017, two *Rhododendron* cultivars were used, *Rhododendron* ‘Fen Zhenzhu’ and *Rhododendron* ‘Zhuangyuan Hong’, which were 3-year-old healthy grown cuttings purchased from Jiashan, Zhejiang province. Plants were grown in plastic pots containing 1:2 (v:v) peat and pine needle mulch, 12 cm high, with a 16-cm diameter at the top and 10 cm at the bottom, in a greenhouse located in Yangling, Shaanxi province. There were five treatments including a control group. Each cultivar had 15 pots and each treatment had three pots for each repeat. Next, 15 pots of each *Rhododendron* cultivar were transferred to a climate chamber (BIC-400, Boxun, Shanghai, China) at 22 °C and light intensity of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photosynthetic photon flux (PPF) with a photoperiod of 14 h day and 10 h night, and 85% relative humidity for 3 days to adapt. After this, 12 pots of each cultivar were transferred to climate chambers under combined stressors, which were a high temperature of 38 °C combined with light intensity of 37.5, 75, 112.5 (double stress treatment), and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (heat stress treatment) for 6 days. The remaining three pots of each cultivar were kept at 22 °C with a light intensity of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ as control (CK). Positions of these pots in the growth chamber were randomly exchanged daily to minimize positional effects. The plants were managed regularly and watered twice a day during the experiment. The plants were sampled after 6 days. The experiment was repeated three times.

2.2 Measurement of relative water content and electrolyte leakage assay

Leaf water status was confirmed by relative water content (RWC; %), calculated as follows: $\text{RWC} = (\text{FW} - \text{DW}) / (\text{SW} - \text{DW}) \times 100$ (Barrs and Weatherley 1962). In this formula, FW is the fresh weight of leaves, DW is the dry weight of leaves after drying at 85 °C for 2 days, and SW is the turgid weight of leaves after being soaked in water for 4 h at 25 °C. Samples (0.1 g) of fresh leaves in different treatments were thoroughly washed in double distilled water, placed in 25 ml double distilled water, and pumped in a vacuum for 30 min. The electrical conductivity was measured by a DDSJ-308A conductivity bridge (Leica, Shanghai, China) after 2 h (EC1). After that, samples were placed in boiling water for 20 min and cooled to room temperature, and the electrical conductivity was again recorded (EC2). The

electrolyte leakage was calculated as $(EC1/EC2 \times 100\%)$ (Palta et al. 1977).

2.3 Measurement of gas exchange characteristics and chlorophyll content

After treatment, gas exchange characteristics were measured on the fully expanded leaf. A portable photosynthesis analysis system (Li-6400XT, LI-COR Lincoln, NE, USA) was used to determine the net photosynthetic rate (P_n), stomatal conductance (G_s), transpiration rate (T_r), and intercellular CO_2 concentration (C_i) between 08:00 a.m. and 10:00 a.m. The system maintained the measurement conditions of 6 cm^2 leaf surface area and $500 \mu\text{mol s}^{-1}$ flow rate to the sample cell.

The leaf chlorophyll content was measured based on the method described by Hiscox and Israelstam (1979). Briefly, fresh leaves (0.1 g) were immersed in 25 ml 80% (v/v) acetone and then the absorbance of the samples at 645 and 663 nm was measured by a UV–Vis spectrophotometer (GENESYS 10S, Thermo Fisher, NY, USA) after 24 h of dark treatment. The chlorophyll content was calculated by the following formulas:

$$\begin{aligned} \text{Chl a (mg/g FW)} &= (12.72 \times A_{663} - 2.59 \times A_{645}) \times V/W, \\ \text{Chl b (mg/g FW)} &= (22.88 \times A_{645} - 4.67 \times A_{663}) \times V/W, \\ \text{Chl total (mg/g FW)} &= (20.2 \times A_{645} + 8.02 \times A_{663}) \times V/W, \end{aligned}$$

where A_{645} and A_{663} are the absorbance of the extract at 645 and 663 nm, respectively, V is the supernatant volume, W is the fresh weight, and FW is the fresh weight of the leaf.

2.4 Measurement of anatomy parameters

Leaf discs measuring $5 \times 5 \text{ mm}$ and $1 \times 10 \text{ mm}$ were sampled from the fully expanded leaves, which were collected between 08:00 a.m. and 10:00 a.m. after treatments for 6 days. The samples were fixed in 4% glutaraldehyde at 4°C for 24 h. After fixation in 4% glutaraldehyde, the samples were washed four times with 0.1 M phosphate buffer (pH 6.8) for 15 min, followed by serial dehydration for 15 min with 30, 50, 70, 80, and 90% (v/v) ethanol, followed by 30 min with 100% (v/v) ethanol before transfer to isoamyl acetate. The samples were then dried with a K-850 CO_2 critical point dryer (Quorum, Laughton, UK) and were mounted on stubs with colloidal silver, sputter-coated with gold using a E-1045 ion coater (Hitachi, Japan) and viewed with a JSM-6360LV scanning electron microscope (JEOL, Tokyo, Japan) at 5 kV. Leaf thickness, cuticle thickness, palisade tissue thickness, and midrib thickness were measured from 15 observations on $1 \times 10\text{-mm}$ leaf discs. The stomatal density and opened stomata ratio were recorded within eight fields of scanning electron microscopy observations on $5 \times 5\text{-mm}$ leaf discs. The length and width

of 15 opened stomata were averaged for the estimation of the aperture according to the equation for the ellipse area: $1/4 \times (\text{length} \times \text{width} \times \pi)$ (Chen et al. 2014).

2.5 Osmoregulation substance analyses

Total soluble sugars were measured according to the method of Irigoyen et al. (1992) with slight modifications. Samples (0.1 g) in different treatments were homogenized in 25 ml double distilled water. The extract was centrifuged at $3500 \times g$ for 10 min. Two milliliters of supernatant was used, and 0.5 ml anthrone reagent and 5 ml concentrated sulfuric acid were added to it and then placed in a boiling water bath for 1 min. After cooling, absorbance of the mixture was recorded at 630 nm using a spectrophotometer. The results were calculated by a standard curve of glucose.

The proline (Pro) content was determined using the acid-ninhydrin method according to Bates et al. (1973). A 0.1-g sample was extracted with 5 ml of 3% aqueous sulfosalicylic acid and placed in a boiling water bath for 10 min. Supernatant (2 ml) was reacted with an equal volume of acid-ninhydrin and glacial acetic acid for 30 min in a test tube placed in a water bath at 100°C and then 5 ml toluene was added. Absorbance of the toluene layer was measured at 520 nm with a spectrophotometer. Proline content was calculated using a standard curve prepared with known concentrations of proline.

The total soluble protein content was measured according to the total soluble protein assay kit (Jiancheng, Nanjing, China), and absorbance of the extract was measured at 595 nm.

2.6 Measurement of malondialdehyde and hydrogen peroxide

The malondialdehyde (MDA) content was measured using a modification of the method reported by Zhou and Leul (1998). Two milliliters of crude leaf extract stored in liquid nitrogen was ground in 10 ml of 10% trichloroacetic acid using a mortar and pestle. The homogenate was centrifuged at $4000 \times g$ for 10 min and then 2 ml supernatant was mixed with 2 ml 0.6% thiobarbituric acid and incubated in boiling water for 15 min. After cooling to room temperature and centrifugation at $4000 \times g$ for 10 min, the absorbance of the supernatant was recorded at 532, 600, and 450 nm with a spectrophotometer. The MDA content was calculated using the following formula:

$$\text{MDA } (\mu\text{mol g}^{-1}) = [6.45 \times (A_{532} - A_{600}) - 0.56 \times A_{450}] \times V/W,$$

where A_{532} , A_{600} , and A_{450} are the absorbance of the extract at 532, 600, and 450 nm, respectively; V represents the supernatant volume and W represents the weight.

The hydrogen peroxide (H_2O_2) content was measured according to the hydrogen peroxide assay kit (Jiancheng,

Nanjing, China). Absorbance of the mixture was recorded at 405 nm with a spectrophotometer.

2.7 Assay of antioxidant enzyme activities

Leaves (0.1 g) were ground in liquid nitrogen and suspended into 2 ml ice-cold PBS buffer (50 mM, pH 7.8). The homogenate was centrifuged at 4 °C and 12,000×g for 20 min, and the resulting supernatants were used to determine the activities of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

SOD activity was measured in accordance with Giannopolitis and Ries (1977). The 3-ml reaction solution contained 13 mM methionine, 75 nM ethylenediaminetetraacetic acid, 50 μM NBT, 1.3 μM riboflavin, 50 mM PBS buffer (pH 7.8), and 50 μl enzyme extract, which was irradiated at 50 μmol m⁻² s⁻¹ for 20 min, and absorbance was measured at 560 nm. The nonirradiated reaction solution was used as a blank. One unit of SOD activity was defined as the amount of enzyme that caused a 50% inhibition of the rate of nitroblue tetrazolium reduction.

POD activity was analyzed as described by Chance and Maehly (1955) with slight modifications, and absorbance was measured at 470 nm. The assay reaction solution contained 50 mM PBS buffer (pH 7.8), 30% (v/v) H₂O₂, 100% guaiacol solution, and 50 μl enzyme extract. A reaction mixture, which contained 22.5 μl enzyme extract, 100 mM phosphate buffer (pH 7.8), and 10 mM H₂O₂, was used to assay the activity of CAT at 240 nm via the decomposing of H₂O₂ ($\epsilon = 40 \text{ M}^{-1} \text{ cm}^{-1}$) (Pereira et al. 2002). One unit of activity for POD and CAT was defined as the amount of enzymes, which inhibited 1% of degraded 1 μM guaiacol radical and degraded 1 mmol H₂O₂ per minute, respectively.

2.8 Statistical analysis

The experiment was a completely randomized design. Every treatment was performed with three replicates. SPSS 19.0 software (IBM Corp, Armonk, NY, USA) was used to analyze the data, and Duncan's multiple range test was used to compare treatments when the variance analysis showed a significant difference between means at $p \leq 0.05$. Different lowercase letters indicate significant differences between *Rh.* 'Fen Zhenzhu' and *Rh.* 'Zhuangyuan Hong' at the same treatment by the least significant difference at $p \leq 0.05$. Bars represent the means \pm standard errors.

3 Results

3.1 Effects of low light stress and high temperature on appearance, RWC, and electrolytes in two *Rhododendron* cultivars

Compared with CK, plants from the two *Rhododendron* cultivars showed different characteristics of leaf injury, such as water loss, wilting, leaf drying, and so on (Fig. 1). At the same time, RWC decreased with a decrease in light intensity, and there was no significant difference between the two cultivars (Fig. 2a). As for the relative conductivity, there was an upward trend with the decrease of light intensity, and there were obvious differences between the two cultivars (Fig. 2b). According to our results, under combined high temperature and low light stress, the damage of *Rhododendron* plants increased with a decrease in light intensity.



Fig. 1 The appearance of two *Rhododendron* cultivars after 6 days under different treatments. *Note:* The meanings of capital letter and number in the label are as follows. F—*Rh.* 'Fen Zhenzhu';

Z—*Rh.* 'Zhuangyuan Hong'; 1—37.5 μmol m⁻² s⁻¹ PPF, 38 °C; 2—75 μmol m⁻² s⁻¹ PPF, 38 °C; 3—112.5 μmol m⁻² s⁻¹ PPF, 38 °C; 4—150 μmol m⁻² s⁻¹ PPF, 38 °C; 5—150 μmol m⁻² s⁻¹ PPF, 22 °C

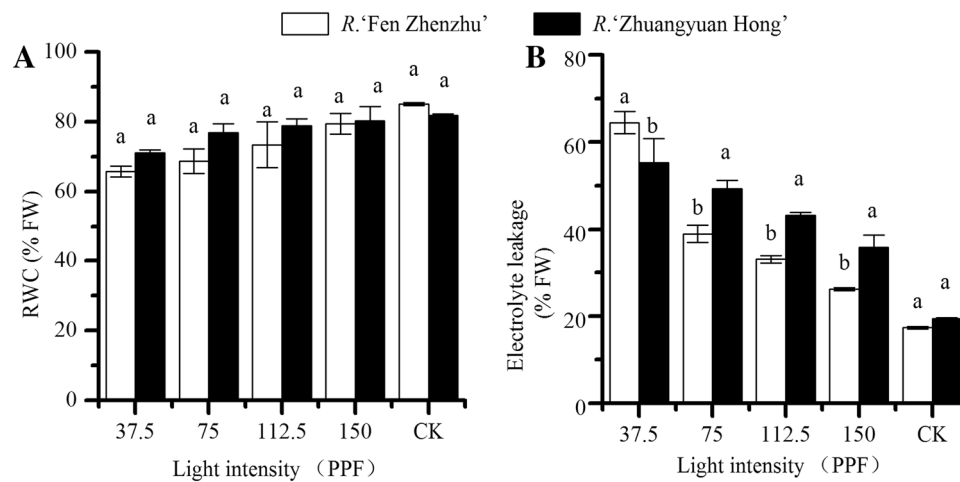


Fig. 2 Effects of low light stress and high temperature on relative water content (a) and electrolyte leakage (b) in two *Rhododendron* cultivars. *Note*: Every treatment was performed with three replicates. Duncan's multiple range test was used to compare treatments when the variance analysis showed a significant difference between means

at $p \leq 0.05$. Different lowercase letters indicate significant differences between Rh. 'Fen Zhenzhu' and Rh. 'Zhuangyuan Hong' at the same treatment by the least significant difference at $p \leq 0.05$. Bars represent the means \pm standard errors

3.2 Effects of low light and high temperature on photosynthesis and chlorophyll content in two *Rhododendron* cultivars

P_n , G_s , and T_r of *Rhododendron* leaves decreased significantly under each treatment compared with the control. There was a significant difference in P_n between the two cultivars when plants were subjected to low light ($112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$), but there was no significant difference in other treatments. Changes in P_n , G_s , and T_r of *Rh.* 'Fen Zhenzhu' leaves decreased the least under single heat stress; P_n and G_s of *Rh.* 'Zhuangyuan Hong' decreased the least under single heat stress, but T_r decreased the least when the light intensity was $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. Under high temperature and low light ($37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$), changes in C_i of the two *Rhododendron* cultivars were not significant (Fig. 3c), but C_i increased the most in the two *Rhododendron* cultivars under $150 \mu\text{mol m}^{-2} \text{s}^{-1}$. C_i of *Rh.* 'Zhuangyuan Hong' leaves reached minimum under $75 \mu\text{mol m}^{-2} \text{s}^{-1}$. According to changes of G_s , C_i , and T_r , the reasons for the decrease in P_n were different for the two *Rhododendron* cultivars under different treatments.

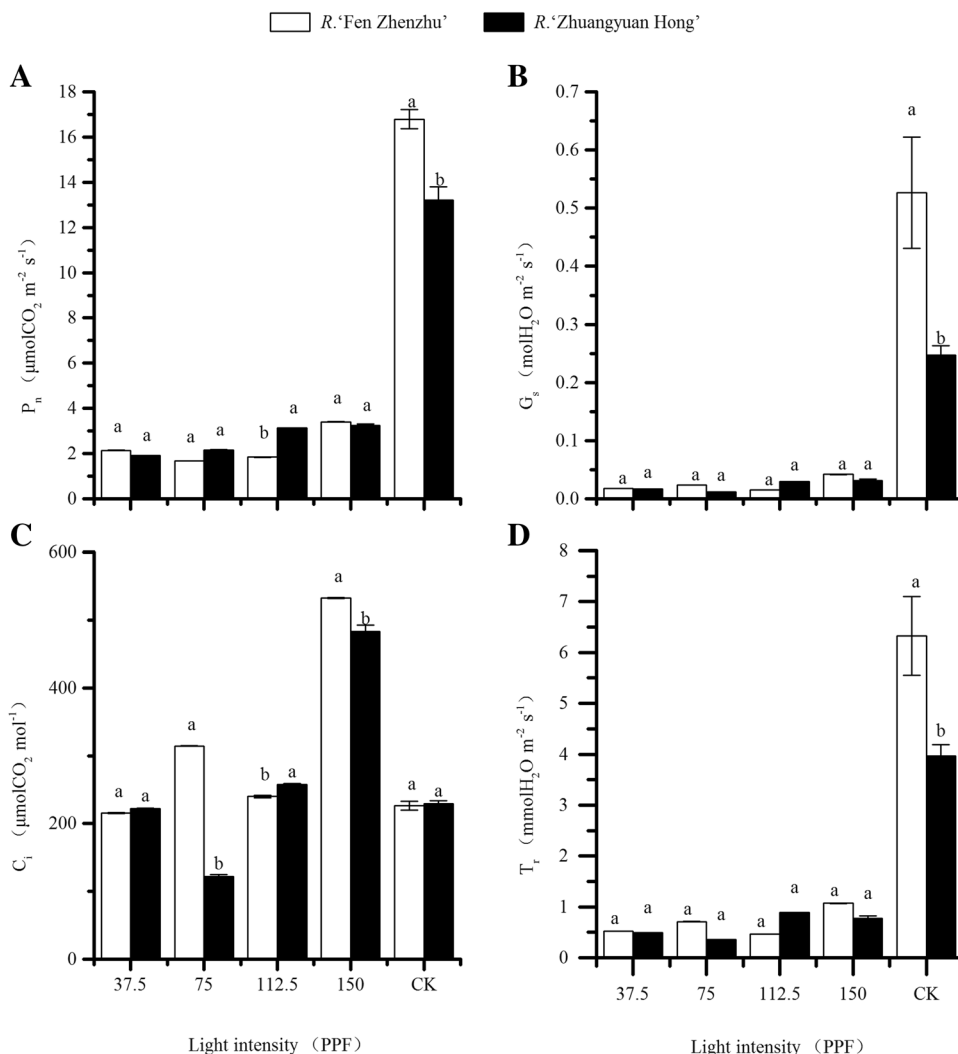
In addition, mass fraction of chlorophyll a, chlorophyll b, and total chlorophyll in the two *Rhododendron* cultivars decreased significantly under stress, and the trend was nearly identical; they both decreased the least under single heat stress (Fig. 4a–c). The ratio of chlorophyll a and chlorophyll b in leaves of *Rh.* 'Fen Zhenzhu' decreased under treatments, but the ratio in leaves of *Rh.* 'Zhuangyuan Hong' increased.

3.3 Effects of low light stress and high temperature on the anatomy of two *Rhododendron* cultivars

Under different treatments, stomatal density and opened stomata ratios of two *Rhododendron* cultivars decreased significantly, while stomatal aperture increased significantly (Figs. 5, 6). Stomatal density and the opened stomata ratio in *Rh.* 'Fen Zhenzhu' decreased the least under low light ($112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$); conversely, *Rh.* 'Zhuangyuan Hong' decreased the most and decreased the least (21.37%) under heat stress (Fig. 6a, b). As for stomatal aperture, there were significant differences between the two cultivars under each treatment. The stomatal aperture of two *Rhododendron* cultivars was the largest under heat stress; it increased the least under low light ($37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 6c).

The anatomic structure of leaves was also affected by high temperature and low light stress (Figs. 7, 8). The ratio of cuticle thickness to leaf thickness of the *Rh.* 'Fen Zhenzhu' leaves was the lowest under low light ($37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) but changed the least under heat stress, while it changed the least in *Rh.* 'Zhuangyuan Hong' under $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF (Fig. 8a). Cell tightness ratio (CTR) in the two *Rhododendron* cultivars decreased compared to that in the control. CTR in *Rh.* 'Fen Zhenzhu' decreased the least under low light ($75 \mu\text{mol m}^{-2} \text{s}^{-1}$), and it changed least in *Rh.* 'Zhuangyuan Hong' when the light intensity was $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 8b). Compared with the control, spongy ratio (SR) of the two cultivars decreased under all treatments. SR in *Rh.* 'Fen Zhenzhu' was not significantly different among different levels of combined stressors with low light; SR of *Rh.* 'Zhuangyuan Hong' decreased the least

Fig. 3 Effects of low light stress and high temperature on net photosynthetic rate (a), stomatal conductance (b), transpiration rate (c), and intercellular CO₂ concentration (d) in two *Rhododendron* cultivars



under low light ($75 \mu\text{mol m}^{-2} \text{s}^{-1}$) (Fig. 8c). The ratio of palisade and sponge tissue was significantly different between *Rh.* 'Fen Zhenzhu' and *Rh.* 'Zhuangyuan Hong' under single heat stress (Fig. 8d). The ratio in leaves of *Rh.* 'Fen Zhenzhu' reached the highest value under $75 \mu\text{mol m}^{-2} \text{s}^{-1}$, while the lowest value was reached in *Rh.* 'Zhuangyuan Hong'. There was no significant difference among these treatments in *Rh.* 'Zhuangyuan Hong'.

3.4 Effects of low light stress and high temperature on osmoregulation substances in two *Rhododendron* cultivars

The content of soluble sugar was significantly different under treatments between the two cultivars, except under combined stress with the light intensity of $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 9a). Soluble sugar in the leaves of *Rh.* 'Fen Zhenzhu' increased except under the light intensity of $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$. Moreover, soluble sugar in leaves of *Rh.* 'Zhuangyuan Hong'

increased under treatments. Under the double stress of high temperature and low light, the soluble protein content of *Rh.* 'Fen Zhenzhu' increased continuously with the decrease of light intensity. Soluble protein of *Rh.* 'Zhuangyuan Hong' increased significantly under double stress but increased the least at light intensity of $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 9b). In comparison to the changes in soluble sugar and soluble protein content, Pro increased compared with the control (Fig. 9c). Pro in *Rh.* 'Fen Zhenzhu' increased the most under low light ($37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$), 5.26 times higher than that of the control. However, there was no significant difference in Pro content of *Rh.* 'Zhuangyuan Hong' before and after stress.

3.5 Effects of low light stress and high temperature on MDA and H₂O₂ in two *Rhododendron* cultivars

The molar concentration of MDA in leaves of two *Rhododendron* cultivars increased under different treatments compared

Fig. 4 Effects of low light stress and high temperature on chlorophyll a (a), chlorophyll b (b), total chlorophyll (c), and chlorophyll a/b (d) in two *Rhododendron* cultivars

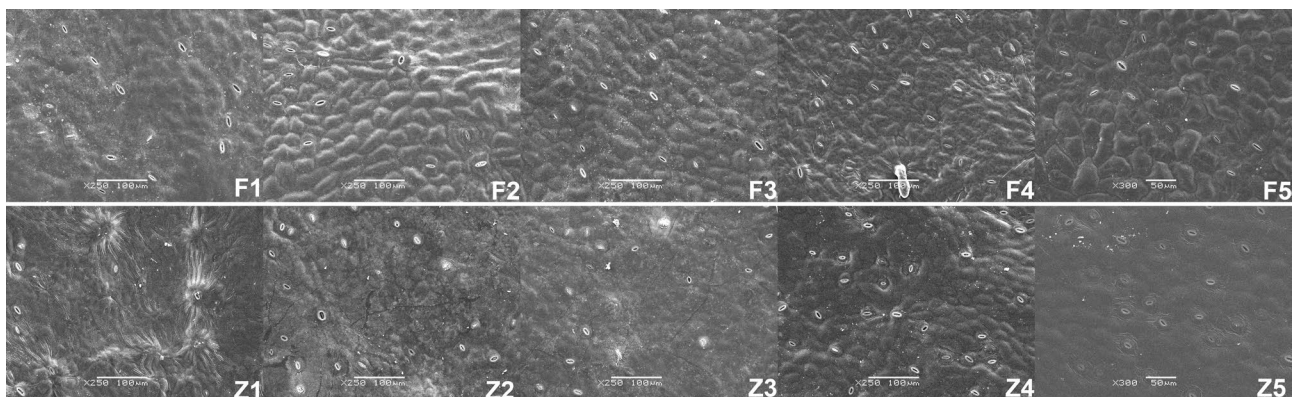
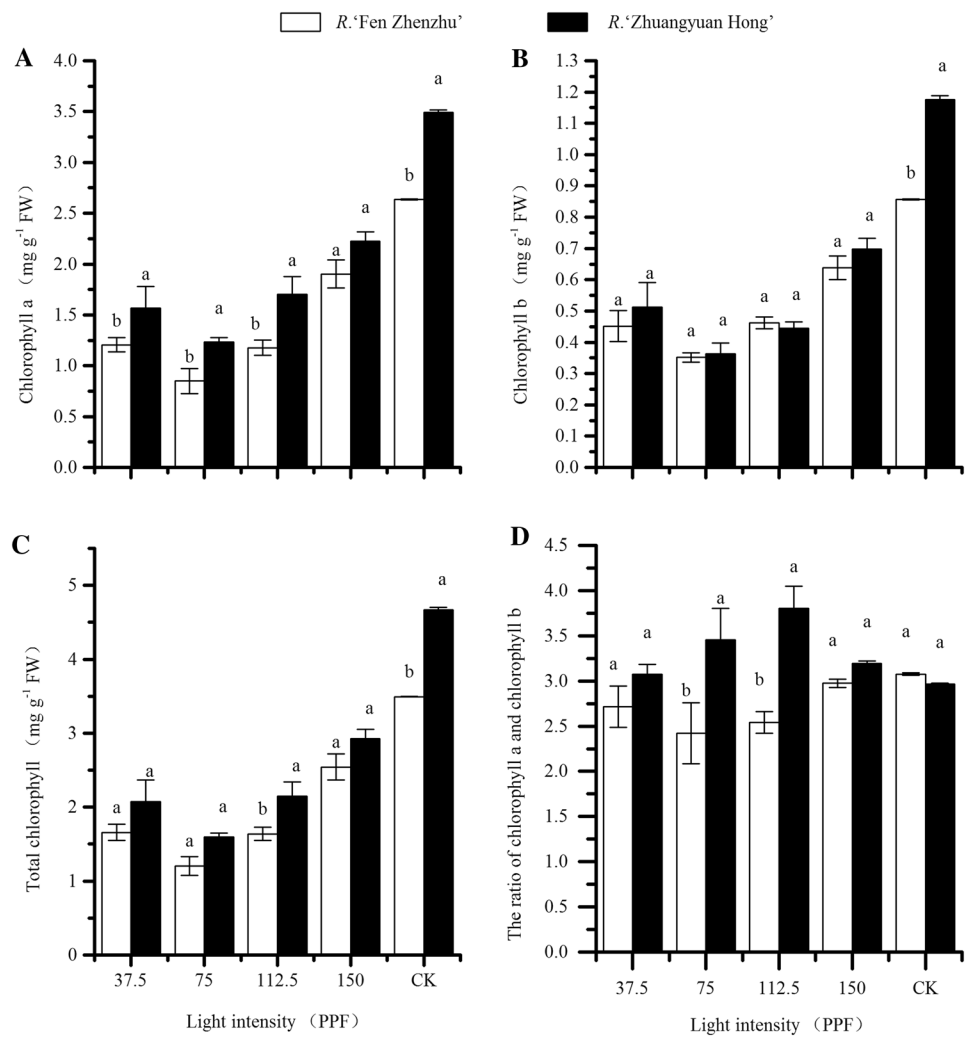


Fig. 5 Leaf stomatal morphology of two *Rhododendron* cultivars after treatments. Note: F—*Rh.* 'Fen Zhenzhu'; Z—*Rh.* 'Zhuangyuan Hong'; 1—37.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 38 °C; 2—75 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF,

38 °C; 3—112.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 38 °C; 4—150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 38 °C; 5—150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 22 °C

with the control (Fig. 10a). The MDA of *Rh.* 'Fen Zhenzhu' increased least but it increased most in *Rh.* 'Zhuangyuan Hong' at a light intensity of 112.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$. As for

the molar concentration of H_2O_2 , there were significant differences between the two cultivars under different stress treatments, but there was no significant difference among

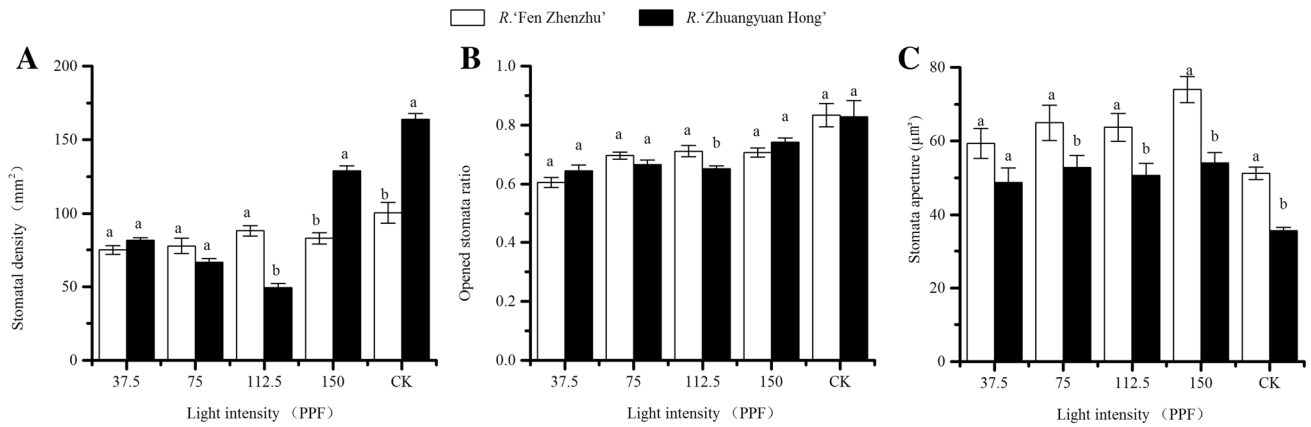


Fig. 6 Effects of low light stress and high temperature on stomatal density (a), opened stomata ratio (b), and stomatal aperture (c) in two *Rhododendron* cultivars. Note: Stomatal density and ratio of opened stomata (the ratio of the average number of open stomata and the

average total number of stomata under eight views) were calculated by eight views of SEM observations. Fifteen opened stomata lengths and widths were averaged to estimate the stomatal area according to the ellipse area formula: $1/4 \times (\text{length} \times \text{width} \times \pi)$

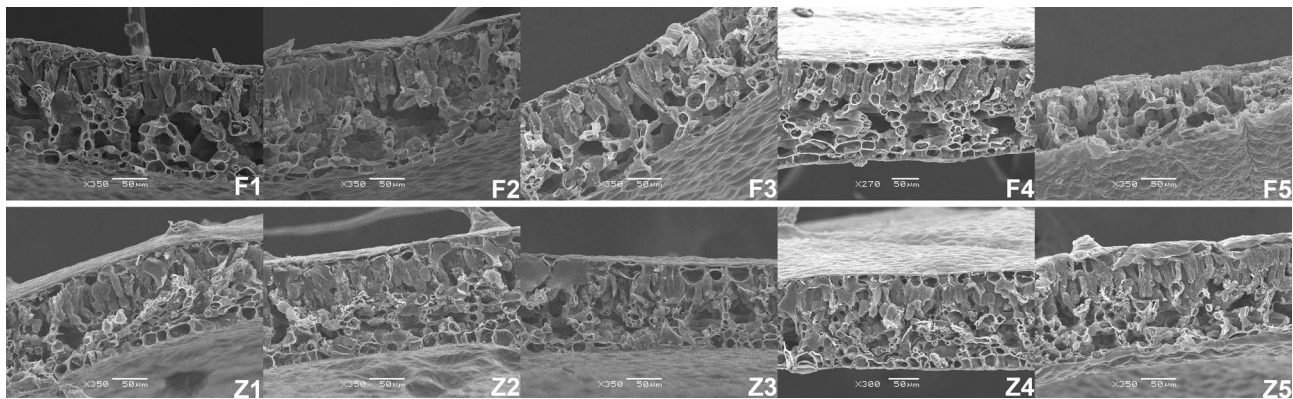


Fig. 7 Leaf morphology of two *Rhododendron* cultivars after treatments. Note: F—*Rh. 'Fen Zhenzhu'*; Z—*Rh. 'Zhuangyuan Hong'*; 1— $37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 38 °C; 2— $75 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 38 °C;

3— $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 38 °C; 4— $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 38 °C; 5— $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF, 22 °C

different treatments in *Rh. 'Zhuangyuan Hong'*. H_2O_2 was highest under $37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ and lowest under $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Rh. 'Fen Zhenzhu'* (Fig. 10b).

3.6 Effects of low light stress and high temperature on ROS scavenging system in two *Rhododendron* cultivars

The three enzyme activities of SOD, POD, and CAT differed in the two cultivars and different treatments (Fig. 11). The SOD activity of leaves was significantly different between two *Rhododendron* cultivars under the same treatment. The SOD activity of *Rh. 'Fen Zhenzhu'* increased significantly

under all treatments and was highest at the light intensity of $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (increased 1.24 times). The activity of SOD in *Rh. 'Zhuangyuan Hong'* decreased significantly under treatments, but the difference was not significant (Fig. 11a). In the two *Rhododendron* cultivars, POD and CAT activities increased under each treatment (Fig. 11b, c). The POD activity was highest under single heat stress in *Rh. 'Fen Zhenzhu'* and under the light intensity of $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Rh. 'Zhuangyuan Hong'*, increasing by 3.33 and 5.49 times, respectively, while CAT activity was highest under single heat stress in *Rh. 'Fen Zhenzhu'* and under the light intensity of $37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ in *Rh. 'Zhuangyuan Hong'*, increasing by 4.17 and 8.92 times, respectively.

Fig. 8 Effects of low light stress and high temperature on cuticle thickness/leaf thickness (a), cell tightness ratio (b), spongy ratio (c), and the ratio of palisade and sponges tissue (d) in two *Rhododendron* cultivars

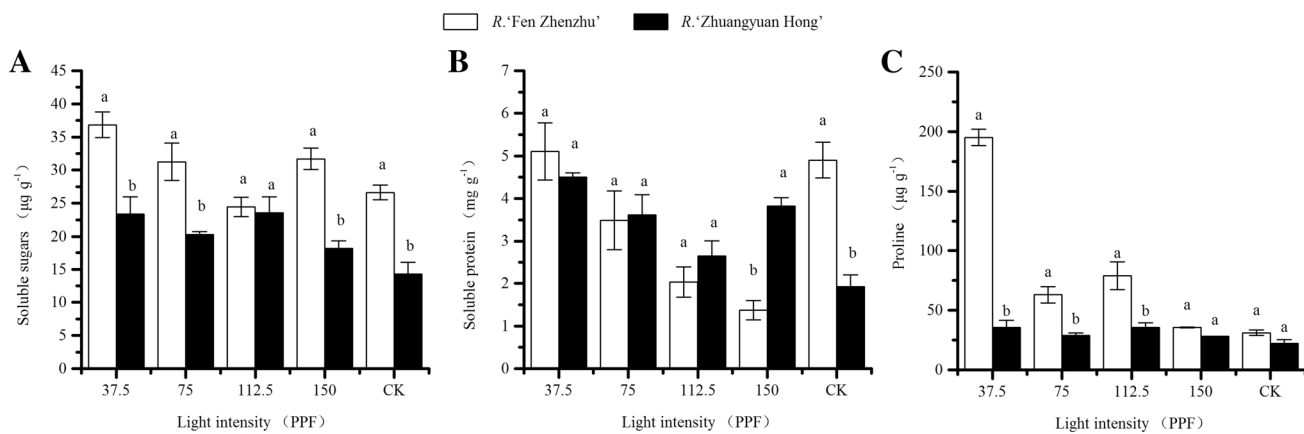
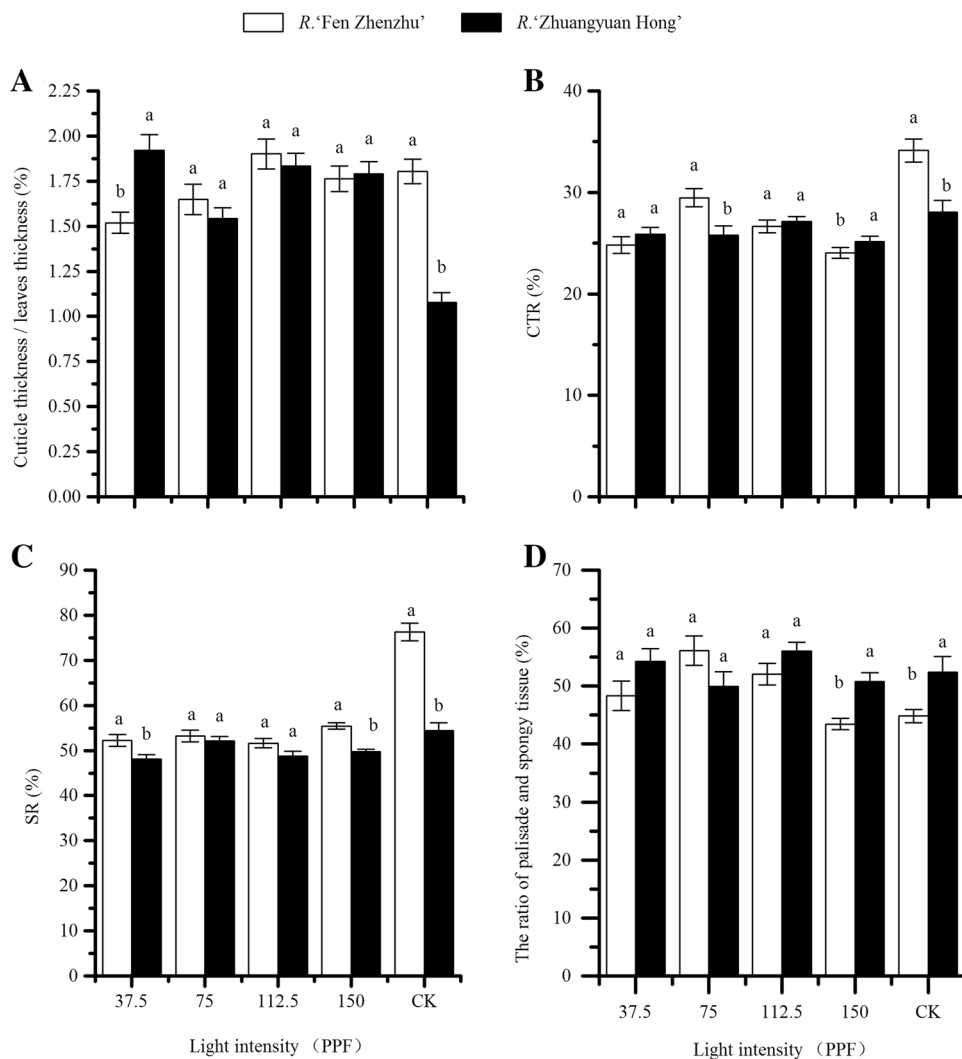


Fig. 9 Effects of low light stress and high temperature on soluble sugar (a), soluble protein (b), and Pro (c) in two *Rhododendron* cultivars

Fig. 10 Effects of low light stress and high temperature on MDA (a) and H₂O₂ (b) in two *Rhododendron* cultivars

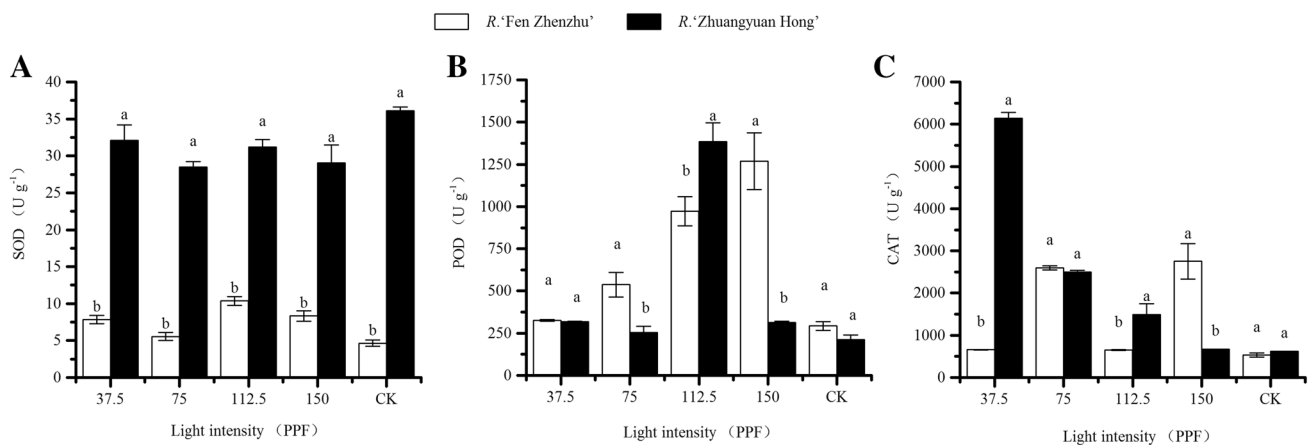
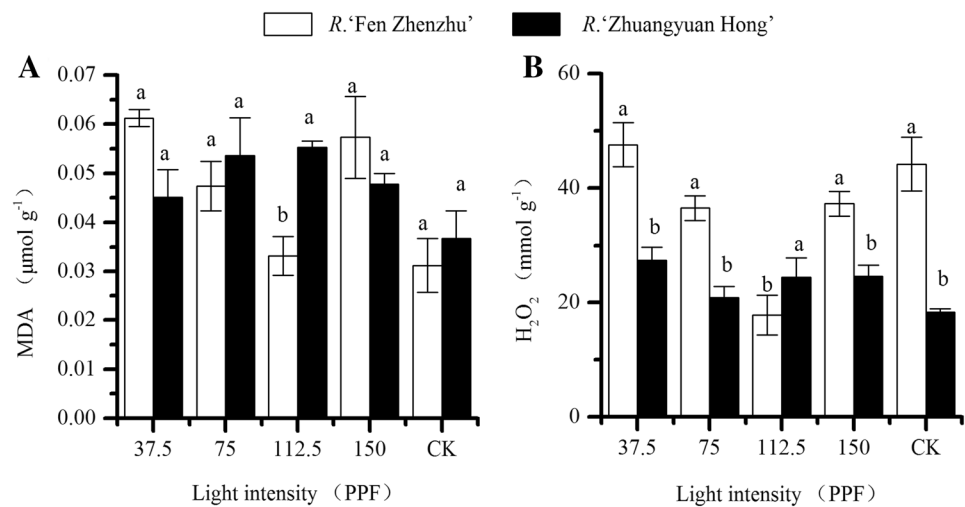


Fig. 11 Effects of low light stress and high temperature on SOD (a), POD (b), and CAT (c) in two *Rhododendron* cultivars

4 Discussion

4.1 Effects of low light and high temperature on the morphology characteristics of two *Rhododendron* cultivars

Light and temperature are important environmental factors that influence the appearance of plant growth. The growth of plants was directly affected by heat stress and low light stress, resulting in water loss, wilting, and curling of leaves (Deng et al. 2012; Chen et al. 2014; Tao et al. 2015; Son et al. 2018). In this experiment, two *Rhododendron* cultivars were injured under different stressors, and owing to the changes in light intensity, the damage that the plants were subjected to differs as well. Under low light intensity (37.5 and 75 μmol m⁻² s⁻¹), the two *Rhododendron* cultivars showed more obvious damage symptoms, and the damage of *Rh.* 'Fen Zhenzhu' was more serious. Ajmi et al. (2018) found that the position of the leaves of shade

plants began to be affected after shading for 3 months. It was more vertical and perpendicular to sunlight, ranging from 67° to 100° to absorb more light. However, the exposed blade position was more horizontal and parallel to the sunlight, ranging from 30° to 60° to avoid excessive incident sunlight. The reason why this phenomenon was not found in the experiment was because the response of plants to shading could be divided into short and long term, and the illumination control time in this experiment was too short. To some extent, plant growth performance is related to leaf water status, and RWC is an important indicator of leaf water status (Jin et al. 2015). Compared with CK, the RWC of leaves of *Rh.* 'Fen Zhenzhu' and *Rh.* 'Zhuangyuan Hong' decreased under high temperature stress, RWC decreased more with an increase in light intensity (Fig. 2a). The results indicated that both high temperature and low light stress could affect the water content of leaves. They also indicated that lower light intensity results in more water loss in leaves and causes

more serious damage. Conversely, the relative conductivity of leaves increased under high temperature stress and increased even more with a decrease in light intensity (Fig. 2b), which is consistent with the study of Liao et al. (2013). The results also showed that the lower light intensity results in more serious damage to leaf cells. At the same time, it can be found that the relative conductivity of the two cultivars is significantly different under different stresses, so the damage mechanism is different. The results of comprehensive growth performance and RWC indicated that lower light exposure would increase the damage of rhododendron under high temperature conditions.

4.2 Effects of low light and high temperature on the anatomical structure of two *Rhododendron* cultivars

When plants are exposed to light and temperature stress, their microstructure and leaf photosynthesis process change to resist the damage. Studies have shown that plants can improve their abilities to capture light energy by changing leaf forms and structure (Chen et al. 2014; Shen et al. 2017; Zhou et al. 2017). At the microstructural level of *Rhododendron* leaves, the ratio of cuticle thickness to leaf thickness in *Rh.* ‘Fen Zhenzhu’ was lowest under combined stress of low light intensity ($37.5 \mu\text{mol m}^{-2} \text{s}^{-1}$) and high temperature but higher than the control under the light intensity of $112.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ (Fig. 8a). The ratio of cuticle thickness to leaf thickness decreased significantly compared with that under high temperature stress, which may be due to the thinness of cuticle formation caused by low light intensity. In comparison to *Rh.* ‘Fen Zhenzhu’, the ratio in *Rh.* ‘Zhuangyuan Hong’ increased significantly under heat stress but changed inconsistently under different levels of low light. This may indicate that for *Rh.* ‘Zhuangyuan Hong’, heat stress or the combined stress of high temperature and low light played a role in promoting cuticle formation or had a negative effect on leaf thickness to some extent, which indirectly led to the increased ratio of cuticle thickness and leaf thickness. At the same time, CTR and SR of leaves of the two *Rhododendron* cultivars decreased under the condition of low light and high temperature (Fig. 8b, c), which was consistent with Deng et al. (2012), who showed that under severe shading or low light, palisade tissue and spongy tissue become smaller in volume and thinner in thickness. The two cultivars may have different self-regulation mechanisms for leaf structure in response to combined stress. Corresponding to changes of CTR and SR, the ratio of palisade and sponges tissue in *Rh.* ‘Zhuangyuan Hong’ was not significantly different when comparing heat stress and combined stress treatments, but *Rh.* ‘Fen Zhenzhu’ increased under the weaker light intensity (Fig. 8d). This differs from the results obtained by Zhou et al. (2017), who showed that

with increasing shade, the ratio of palisade and sponge tissue decreases. At the same time, Ajmi et al. (2018) also found that the thickness of the leaves was significantly reduced by shading, mainly due to the reduction of palisade tissue and spongy parenchyma. At high temperatures, the leaves of the plants are thinner, the thickness of the epidermis, fence, and sponge layer is reduced, and the cell size of the mesophyll is reduced. It is possible that because the combined stresses caused fewer effects on anatomical structure than shading and low light, some of the damage under high temperature was relieved to some extent in this study. Between the two cultivars, CTR and SR of *Rh.* ‘Zhuangyuan Hong’ and the ratio of palisade and sponges tissue changed little among the treatments, which indicated that the leaf structure of *Rh.* ‘Zhuangyuan Hong’ was more stable than that of *Rh.* ‘Fen Zhenzhu’ when subjected to stressors.

Photosynthesis is considered to be the most vulnerable to heat stress and low light stress in plants (Kim et al. 2011; Lu et al. 2017). High temperatures have an adverse effect on plant growth. Temperature higher than the optimum temperature, the chloroplast structure will be destroyed and photosynthesis will be decreased (Chen et al. 2012). Long-term exposure of the leaves to lower light intensities may also alter related photosynthetic parameters such as stomatal density, stomatal conductance, and chlorophyll content (Ajmi et al. 2018). The results showed that P_n of *Rh.* ‘Fen Zhenzhu’ and *Rh.* ‘Zhuangyuan Hong’ decreased significantly under low light, which suggests that low light or combined stress might have a negative effect on the net photosynthesis of *Rhododendron* plants. However, Pang et al. (2019) indicated that plants grown under moderate shading conditions might achieve maximum net assimilation for most of the time, while plants grown without shading absorb excess light, resulting in high dark respiration rates and low photosynthetic efficiency. To some extent, changes in chlorophyll content can reflect changes of P_n (Yao et al. 2017). Results also proved that the decrease of P_n was closely related to the decrease of chlorophyll mass fraction under combined stress. The ratio of chlorophyll a and b is one of the important indexes of shading tolerance in plants, and its reduction, which is beneficial to low light use, was more due to the increase of chlorophyll b than chlorophyll a. This is likely due to light interception and a change in the organization of both light-intercepting and electron transport components (Yao et al. 2017). In *Rh.* ‘Fen Zhenzhu’, under heat stress and low light, the ratio of chlorophyll a and b decreased with a decrease in light intensity, which showed that low light was used effectively under these stress conditions. Similar results were found by Ajmi et al. (2018). However, the ratio of chlorophyll a and b in *Rh.* ‘Zhuangyuan Hong’ increased under combined stress, indicating that the ability of utilizing low light in *Rh.* ‘Zhuangyuan Hong’ may be lower than that of *Rh.* ‘Fen Zhenzhu’ and that the plants’

shade tolerance may be low. Photosynthesis reduction can be divided into stomatal limitation and nonstomatal limitation. Stomatal factors generally include G_s , C_i , and T_r , and the decrease of G_s resulted in a decrease in P_n (Yu et al. 2015). While the decrease in G_s does not necessarily reduce P_n under high temperature stress, and the nonstomatal limitation may also lead to the reduction of P_n by destroying the structure and function of the chloroplast, excessively producing ROS, and reducing Rubisco efficiency (Hao et al. 2019). The former results showed that the change trend of C_i and P_n is the main basis for judging stomatal or nonstomatal factors. When the change trend of C_i and P_n is the same (that is, there is a positive correlation between C_i and P_n), the decrease of P_n is mainly caused by stomatal factors, and when the change trend of C_i and P_n is opposite (that is, there is negative correlation between them), it is suggested that nonstomatal limiting factors (photosynthetic pigment and photosynthetic enzyme) play a greater role. The nonstomatal limitation of photosynthesis may also be attributed to the decline in Rubisco efficiency under high temperature stress (Hao et al. 2019). The C_i of *Rh.* ‘Fen Zhenzhu’ increased under low light (75, 112.5, and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$), while the C_i of *Rh.* ‘Zhuangyuan Hong’ increased under the light intensity of 112.5 and 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which suggests that the nonstomatal factors influenced P_n . Stomata are the channels for controlling gas flow and water exchange in plant leaves, and the density of stomata is greatly affected by light and heat. Typically, the density of stomata decreases with a decrease in light intensity (Zhou et al. 2017). In this study, the stomatal density and opened stomata ratio of both *Rhododendron* cultivars decreased under stress, which was consistent with the changes in T_r and G_s . However, stomatal aperture increased under stress, which may be a measure for *Rhododendron* plants to tackle high temperature and low light stress.

4.3 Effects of low light and high temperature on the physiology response of two *Rhododendron* cultivars

An important adaptive mechanism of plant resistance to stress is the accumulation of osmotic regulatory substances. The accumulation of these substances, such as soluble sugar, soluble protein, and Pro, can reduce cell swelling and maintain osmotic balance or participate in cell redox reaction to reduce ROS production and increase plant resistance to stress (Deng et al. 2012; Mirza et al. 2013; Shen et al. 2017; Han et al. 2018). The results of our study showed that the changes in osmotic regulators in *Rhododendron* leaves were related to cultivars and stress treatments. The results indicated the two cultivars have lower soluble sugar and soluble protein content and higher Pro content under moderate illumination. Studies have shown that plants grown

at high temperatures consume more energy to avoid harmful effects on plant tissue structure and function (Hao et al. 2019). The decrease of total soluble sugar concentration in shading leaves may be caused by the decrease of photosynthesis under shading conditions (Ajmi et al. 2018). Soluble sugar and Pro contents increased significantly under low light (37.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in *Rh.* ‘Fen Zhenzhu’, which demonstrated that under low light and high temperature, leaves mainly depend on these two substances to regulate cell osmotic potential, while under the single stress of high temperature, it mainly depends on the increase of soluble protein to regulate the cell osmotic potential. *Rh.* ‘Zhuangyuan Hong’ mainly depended on soluble sugar and soluble protein to help regulate cell osmotic potential, which indicates that the mechanism of osmotic regulation of two *Rhododendron* cultivars differs. The results of our study showed that the changes in osmotic regulators in *Rhododendron* leaves were related to cultivars and stress treatments.

MDA is the final product of membrane lipid peroxidation, which damages the integrity of the cell membrane and increases cell membrane permeability (Deng et al. 2012; Jin et al. 2015). The results of our study showed that the largest increase in MDA happened under 37.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in *Rh.* ‘Fen Zhenzhu’, while in *Rh.* ‘Zhuangyuan Hong’, MDA increased most under the light intensity of 112.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$, which indicated that the damage to the leaf cells was most serious under these stress conditions. Stress also leads to the accumulation of ROS, and increases the activity and yield of active oxygen scavenging enzymes, which can rapidly scavenge superoxide anion radicals to reduce the damage to cells (Ding et al. 2016). For instance, SOD can convert superoxide into H_2O_2 to resist ROS stress, and the generated H_2O_2 is further eliminated by POD and CAT (Deng et al. 2012, 2018; Lu et al. 2017; Han et al. 2018). In this experiment, the molar concentration of H_2O_2 in *Rh.* ‘Fen Zhenzhu’ was highest under the light intensity of 37.5 $\mu\text{mol m}^{-2} \text{s}^{-1}$ but was even lower under other treatments than those of the control. This may be attributed to the high activity of POD and CAT under these treatments, resulting in the removal of most of H_2O_2 transformed by SOD. Conversely, it was found that the concentration of H_2O_2 in *Rh.* ‘Zhuangyuan Hong’ was consistent with a change in SOD activity, which indicates that the superoxide could be transformed into H_2O_2 by SOD to resist ROS stress.

Our results show that the combined effects of high temperature and low light stress could cause damage to leaves through various mechanisms, such as loss of water, damage to the cell membrane, decreased net photosynthetic rate, and changes to the leaf structure. With decreased light intensity, damage was aggravated. Different damage mechanisms were found in the two different cultivars and treatments. This study revealed that though plants of *Rhododendron* are shade tolerant, they are not heat tolerant. Additionally, our results

suggest that it is not that the lower the light intensity, the better the growth and performance of the plant. Therefore, in the production of *Rhododendron* in summer, more attention should be paid to offer moderate (rather than excessive) shading to plants to avoid combined stress, which increases the degree of injury.

Acknowledgements We are all grateful for the funding from the Shaanxi Provincial Natural Science Foundation (2018JQ3060) and the basic research business expenses (Z109021703).

Author's contribution BZ put forwarded the concept, ensured the feasibility and rationality of the experiment, and also proofread the manuscript. WL and YL designed and carried out the experiments, analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

Data availability The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Supplementary data Supplementary data as shown in the figures.

Compliance with ethical standards

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

References

- Ajmi A, Vázquez S, Morales F, Chaari A, El-Jendoubi H, Abadía A, Larbi A (2018) Prolonged artificial shade affects morphological, anatomical, biochemical and ecophysiological behavior of young olive trees (cv. Arbosana). *Sci Hortic* 241:275–284. <https://doi.org/10.1016/j.scienta.2018.06.089>
- Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity techniques for estimating water deficits in leaves. *Aust J Biol Sci* 15:413–428. <https://doi.org/10.1071/BI9620413>
- Bates LE, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207. <https://doi.org/10.1007/BF00018060>
- Bitá CE, Tom G (2013) Plant tolerance to high temperature in a changing environment: scientific fundamentals and production of heat stress-tolerant crops. *Front Plant Sci* 4:273. <https://doi.org/10.3389/fpls.2013.00273>
- Chance B, Maehly AC (1955) Assay of catalase and peroxidases. *Method Enzymol* 2:764–775. [https://doi.org/10.1016/S0076-6879\(55\)02300-8](https://doi.org/10.1016/S0076-6879(55)02300-8)
- Chen WR, Zheng JS, Li YQ, Guo WD (2012) Effects of high temperature on photosynthesis, chlorophyll fluorescence, chloroplast ultrastructure, and antioxidant activities in fingered citron. *Russ J Plant Physiol* 59:732–740. <https://doi.org/10.1134/S1021443712060040>
- Chen WL, Yang WJ, Lo HF, Yeh DM (2014) Physiology, anatomy, and cell membrane thermostability selection of leafy radish (*Raphanus sativus* var. *oleiformis* Pers.) with different tolerance under heat stress. *Sci Hortic* 179:367–375. <https://doi.org/10.1016/j.scienta.2014.10.003>
- Christiaens A, Lootens P, Roldan-Ruiz I, Pauwels E, Gobin B, Van LMC (2014) Determining the minimum daily light integral for forcing of azalea (*Rhododendron simsii*). *Sci Hortic* 177:1–9. <https://doi.org/10.1016/j.scienta.2014.07.028>
- Deng YM, Shao QS, Li CC, Ye XQ, Tang RS (2012) Differential responses of double petal and multi petal jasmine to shading: II. Morphology, anatomy and physiology. *Sci Hortic* 144:19–28. <https://doi.org/10.1016/j.scienta.2012.06.031>
- Deng YM, Jia XP, Sun XB, Liang LJ, Su JL (2018) Comparison of jasmine antioxidant system responses to different degrees and durations of shade. *Acta Physiol Plant* 40:41. <https://doi.org/10.1007/s11738-018-2618-7>
- Ding XT, Jiang YP, Hao T, Jin HJ, Zhang HM, He LZ, Zhou Q, Huang DF, Hui DF et al (2016) Effects of heat shock on photosynthetic properties, antioxidant enzyme activity, and downy mildew of cucumber (*Cucumis sativus* L.). *PLoS ONE* 11:e0152429
- Giannopolitis CN, Ries SK (1977) Superoxide dismutases: I. Occurrence in higher plants. *Plant Physiol* 59:309–314. <https://doi.org/10.1104/pp.59.2.309>
- Grover A, Mittal D, Negi M, Lavania D (2013) Generating high temperature tolerant transgenic plants: achievements and challenges. *Plant Sci* 205–206:38–47. <https://doi.org/10.1016/j.plantsci.2013.01.005>
- Gu K, Geng XM, Yue Y, Ozaki Y (2016) Contribution of keeping kore stable anatomical structure under high temperature to heat resistance of *Rhododendron* seedlings. *J Fac Agric Kyushu Univ* 61:273–279
- Han CJ, Wang Q, Zhang HB, Wang SH, Song HD, Hao JM, Dong HZ (2018) Light shading improves the yield and quality of seed in oil-seed peony (*Paeonia ostii* Feng Dan). *J Integr Agric* 17:1631–1640. [https://doi.org/10.1016/S2095-3119\(18\)61979-3](https://doi.org/10.1016/S2095-3119(18)61979-3)
- Hao LH, Guo LL, Li RQ, Cheng Y, Huang L, Zhou HR, Xu M, Li F, Zhang XX et al (2019) Responses of photosynthesis to high temperature stress associated with changes in leaf structure and biochemistry of blueberry (*Vaccinium corymbosum* L.). *Sci Hortic* 246:251–264. <https://doi.org/10.1016/j.scienta.2018.11.007>
- Hiscox JT, Israelstam G (1979) A method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57:1332–1334. <https://doi.org/10.1139/b79-163>
- Ihuyen DTT, Mizunoe Y, Van DT, Miyajima I (2018) Heat stress tolerance in *Rhododendron simsii* Planch. *J Fac Agric Kyushu Univ* 63:241–247
- Irigoyen JJ, Emerich DW, Sanchez-Diaz M (1992) Water stress induced changes in the concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiol Plant* 8:455–460. <https://doi.org/10.1111/j.1399-3054.1992.tb08764.x>
- Jin R, Wang Y, Liu R, Gou J, Chan Z (2015) Physiological and metabolic changes of purslane (*Portulaca oleracea* L.) in response to drought, heat, and combined Stresses. *Front Plant Sci* 6:1123. <https://doi.org/10.3389/fpls.2015.01123>
- Kim SJ, Yu DJ, Kim TC, Lee HJ (2011) Growth and photosynthetic characteristics of blueberry (*Vaccinium corymbosum* cv. Bluecrop) under various shade levels. *Sci Hortic* 129:486–492. <https://doi.org/10.1016/j.scienta.2011.04.022>
- Kondratovics R, Kondratovics U (2017) Introduction and breeding of *Rhododendron* in Latvia. *Proc Latvian Acad Sci Sect B* 71:248–252. <https://doi.org/10.1515/prolas-2017-0041>
- Liao J, Zhou H, Liu B, Xia YP (2013) Influence of different shading on leaf temperature and physiological response to the summer high temperature of *Rhododendron pulchrum*. *North Hortic* 7:74–77
- Lu T, Meng ZJ, Zhang GX, Qi MF, Sun ZP, Liu YF, Li TL (2017) Sub-high temperature and high light intensity induced irreversible inhibition on photosynthesis system of tomato plant (*Solanum lycopersicum* L.). *Front Plant Sci* 8:365. <https://doi.org/10.3389/fpls.2017.00365>
- Mirza H, Kamrun N, Md Mahabub A, Rajib R, Masayuki F (2013) Physiological, biochemical, and molecular mechanisms of heat

- stress tolerance in plants. *Int J Mol Sci* 14:9643–9684. <https://doi.org/10.3390/ijms14059643>
- Pang K, Van Sambeek JW, Navarrete-Tindall NE, Lin CH, Jose S, Garrett HE (2019) Responses of legumes and grasses to non-, moderate, and dense shade in Missouri, USA. I. Forage yield and its species-level plasticity. *Agrofor Syst* 93:11–24. <https://doi.org/10.1007/s10457-017-0067-8>
- Palta JP, Levitt J, Stadelman E (1977) Freezing injury in onion bulb cells. I. Evaluation of the conductivity method and analysis of ion and sugar efflux from injured cells. *Plant Physiol* 60:393–397. <https://doi.org/10.1104/pp.60.3.393>
- Pereira GJG, Molina SMG, Lea PJ, Azevedo RA (2002) Activity of antioxidant enzymes in response to cadmium in *Crotalaria juncea*. *Plant Soil* 239:123–132. <https://doi.org/10.1023/A:1014951524286>
- Reinhardt K, Smith WK (2016) Chlorophyll fluorescence and photosynthetic gas exchange under direct versus diffuse light in evergreen conifer (*Picea pungens*) shoots and broadleaf shrub (*Rhododendron ponticum*) leaves. *Plant Ecol* 217:443–450. <https://doi.org/10.1007/s11258-016-0586-9>
- Shen HF, Zhao B (2018) Study on evaluation of heat tolerance and its physiological mechanisms in *Rhododendron* cultivars. *Plant Physiol J* 54:335–345
- Shen HF, Zhao B, Xu JJ, Liang W, Huang WM, Li HH (2017) Effects of heat stress on changes in physiology and anatomy in two cultivars of *Rhododendron*. *S Afr J Bot* 112:338–345. <https://doi.org/10.1016/j.sajb.2017.06.018>
- Son IC, Moon KH, Song EY, Wi SH, Seo HH, Moon YE, Reddy V, Yang J, Sicher R, Oh S (2018) Growth and physiological responses of Chinese cabbage to different light intensity until leafy head formation. *Hortic Sci Technol* 36:151–160. <https://doi.org/10.12972/kjhst.20180016>
- Soto A, Hernández L, Quiles MJ (2014) High root temperature affects the tolerance to high light intensity in *Spathiphyllum* plants. *Plant Sci* 227:84–89. <https://doi.org/10.1016/j.plantsci.2014.07.004>
- Tao QJ, Wu YY, Fu T, Xiang XN, Li B (2015) Effect of low light stress on physiological characteristics and ultrastructure of *Rhododendron hybridum* leaves. *Sci Silv Sin* 51:84–92. <https://doi.org/10.11707/j.1001-7488.20150311>
- Vainola A, Junttila O (1998) Growth of *Rhododendron* cultivars as affected by temperature and light. *J Hortic Sci Biotechnol* 73:812–821. <https://doi.org/10.1080/14620316.1998.11511053>
- Vasseur F, Pantin F, Vile D (2011) Changes in light intensity reveal a major role for carbon balance in *Arabidopsis* responses to high temperature. *Plant Cell Environ* 34:1563–1576. <https://doi.org/10.1111/j.1365-3040.2011.02353.x>
- Wu JW, Li JY, Su Y, He Q, Wang JH, Qiu Q, Ma JW (2017) A morphophysiological analysis of the effects of drought and shade on *Catalpa bungei* plantlets. *Acta Physiol Plant* 39:80. <https://doi.org/10.1007/s11738-017-2380-2>
- Yao XD, Li CH, Li SY, Zhu Q, Zhang HJ, Wang HY, Yu CM, Steven KSM, Xie FT (2017) Effect of shade on leaf photosynthetic capacity, light-intercepting, electron transfer and energy distribution of soybeans. *Plant Growth Regul* 83:409–416. <https://doi.org/10.1007/s10725-017-0307-y>
- Yu HY, Murchie EH, Gonzalez-Carranza ZH, Pyke KA, Roberts JA (2015) Decreased photosynthesis in the erect panicle 3 (ep3) mutant of rice is associated with reduced stomatal conductance and attenuated guard cell development. *J Exp Bot* 66:1543–1552. <https://doi.org/10.1093/jxb/eru525>
- Zhang Q, Zhang TJ, Chow WS, Xie X, Chen YJ, Peng CL (2015) Photosynthetic characteristics and light energy conversions under different light environments in five tree species occupying dominant status at different stages of subtropical forest succession. *Funct Plant Biol* 42:609–619. <https://doi.org/10.1071/FP14355>
- Zhou WJ, Leul M (1998) Uniconazole-induced alleviation of freezing injury in relation to changes in hormonal balance, enzyme activities and lipid peroxidation in winter rape. *Plant Growth Regul* 26:41–47. <https://doi.org/10.1023/A:100600492>
- Zhou Y, Huang LH, Wei XL, Zhou HY, Chen X (2017) Physiological, morphological, and anatomical changes in *Rhododendron agastum* in response to shading. *Plant Growth Regul* 81:23–30. <https://doi.org/10.1007/s10725-016-0181-z>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.