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Photosynthetic acclimation of rhododendrons to light intensity in relation to leaf water-related traits

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Abstract Leaves under high light may suffer from risks caused by excessive light energy and dehydration. However, it remains unclear how leaf water-related traits affect the photosynthetic acclimation of plants to light intensity. We investigated the photosynthesis and leaf water-related traits of two hybrids (*Rhododendron* 'XXL' and 'Furnivall's daughter') and one native species (*Rhododendron delavayi*) under different growth irradiances to determine the importance of leaf water-related traits on the photosynthetic acclimation of rhododendrons to irradiance. Compared to plants under low light, the values for light saturation point (LSP), stomatal density (SD), leaf dry mass per unit area, and vein density (D_v) in the three rhododendrons were higher under high light, but the values for chlorophyll content and leaf size were lower. There were significant differences in light-saturated photosynthetic rate (P_{max}), stomatal conductance (g_s), transpiration rate (T_r), and non-photochemical quenching (NPQ) in *R. delavayi* and *Rhododendron* 'XXL' between plants grown under high and low irradiance levels, but not in 'Furnivall's daughter'. The positive correlations of D_v and SD with P_{max}, T_r, and LSP indicate that the increase in water transport capacity can improve water supply to meet transpirational demand and regulate photosynthetic performance under high irradiance. Meanwhile, the decrease in chlorophyll content and the increase in NPQ prevent the photodamage of photosystem II by decreasing light absorption and enhancing heat dissipation, respectively, when the plants are exposed to high light. These results suggest that adjusting leaf water-related traits and reducing the damage on photosystem II caused by excess light play important roles in the photosynthetic acclimation of rhododendrons to growth irradiance.

Keywords *Rhododendron* · High-light stress · Stomatal density · Vein density · Photosynthesis

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Introduction

The acclimation of plants to light intensity has been widely investigated across species and habitats (Evans and Poorter 2001; Reinhardt and Smith 2016; Zhang et al. 2017), since sunlight is one of the major environmental factors that influences photosynthesis and growth. Both insufficient light and excess light energy can inhibit photosynthesis efficiency and carbon gain. To optimize light absorption and photosynthetic efficiency, plants can acclimate to their growth irradiances at various levels ranging from whole-plant morphology to the components of the photosynthetic apparatus (Evans and Poorter 2001; Rozendaal et al. 2006; Niinemets 2007; Athanasiou et al. 2010). Under low light conditions, plants may increase light capture by various approaches, such as increasing leaf area and chlorophyll content, and adjusting the exposure of the leaves toward incident irradiance (Givnish 1988; Hikosaka and Terashima 1995; Niinemets 2007). Contrarily, plants grown in high light tend to have smaller leaves with higher nitrogen content, more photosynthetic enzymes, less chlorophyll per unit nitrogen, increased electron transport capacity per unit chlorophyll, and a greater ratio of electron transport capacity to Rubisco activity than in low light (Givnish 1988; Evans and Poorter 2001; Athanasiou et al. 2010). However, the excess light energy can depress photosynthetic efficiency, mainly due to oxidative damage to the photosystem II (PSII) (Long et al. 1994; Kato et al. 2003). Accordingly, plants have developed numerous strategies to adjust light capture and minimize the risk of damage arising from the over-excitation of the photosynthetic apparatus, including increased thermal dissipation, efficient degradation of photo-damaged proteins, and scavenging of active oxygen species (Xu et al. 2009; Kato et al. 2003). Both photochemical quenching and non-photochemical quenching protect the photosynthetic apparatus by dissipating excitation energy as heat, which minimizes production of reactive oxygen species in the PSII antenna (Demmig-Adams and Adams 1992; Müller et al. 2001). In addition to the direct effects mentioned above, light intensity can also affect photosynthesis by changing leaf temperature and water status. Previous studies have found that the increase in light intensity may increase leaf temperature and transpiration rate (Raimondo et al. 2009; Kim et al. 2011), and plants in sunny habitats has high water demand (Nardini et al. 2005). Leaves under high irradiance can be exposed to simultaneous heat, water, and high-light stresses (Niinemets 2007). These imply that the response to water status may play an important role in photosynthetic acclimation to high light (Hanba et al. 2002). However, there is a lack of information on how leaf hydraulic traits plastically respond to different light intensities (Scoffoni et al. 2015), and how leaf water-related traits are adjusted to affect photosynthetic acclimation of plants to growth irradiance.

Growth irradiance can affect water vapor pressure deficit (VPD) and evaporative demand (Raimondo et al. 2009; Kim et al. 2011), therefore plant water relationships (Fetcher et al. 1983; Scoffoni et al. 2015). Both leaf temperature and VPD scale positively with light intensity (Niinemets 2007; Barman et al. 2008). Thus, leaves under high light may suffer from risks caused by high VPD and dehydration. Previous studies have suggested that the traits related to leaf morphology and anatomy play important roles in the adaptation of plants to growth irradiances (Rozendaal et al. 2006; Murphy et al. 2012; Scoffoni et al. 2015). A number of morphological and anatomical traits are considered as ‘drivers’ of leaf hydraulics, such as leaf size, vein density, stomatal density, and width and number of conduits (Sack & Frole 2006; Brodribb and Jordan 2011; Murphy et al. 2014). Under high irradiance, plants tend to have smaller leaves, and thus allow leaves to have thinner boundary layers to reduce their heat loads (Givnish 1988; Vogel 2009). The potential transpirational demand is primarily determined by stomatal aperture and density, while leaf venation aids in replacing the water transpired during photosynthetic gas exchange (Sack and Frole 2006; Brodribb and Jordan 2011). Vein density is correlated with stomatal density, maximum hydraulic conductance, and photosynthetic rate (Sack and Frole 2006; Brodribb and Jordan 2011). Species coping with high water demand in sunny habitats may have an efficient conducting system to supply mesophyll cells with water (Nardini et al. 2005). Shade leaves have bigger stoma, but tend to have lower vein and stomatal densities (Kim et al. 2011; Brodribb and Jordan 2011). However, these patterns are not uniform across species. In some species, stomatal density and vein density do not vary under different light intensities (Fetcher et al. 1983; Amiard et al. 2005). The response pattern of leaf hydraulic conductance to varying light intensities is highly species dependent (Xiong et al.

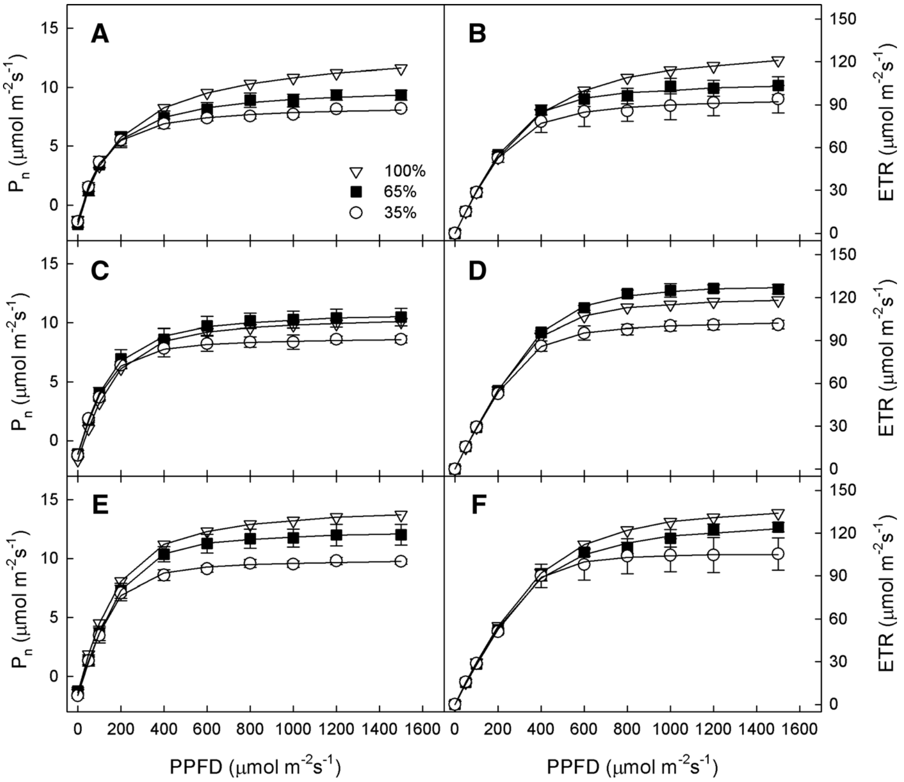

Fig. 1 Responses of photosynthetic rates (P_n) and electron transport rates of PSII (ETR) to photosynthetic photon flux density (PPFD) in rhododendrons grown under different irradiances. **a**, **b** *Rhododendron* 'XXL'; **c**, **d** *Rhododendron* 'Furnivall's daughter'; **e**, **f** *Rhododendron delavayi*. Each point represents mean ± SE of 3 measurements from different plants

2018). Thus, further research is needed to understand the effect of leaf water-related traits on photosynthetic acclimation to growth irradiance.

Rhododendron is one of the most important woody ornamental plants in the world, and is widely used in garden and urban landscapes or as potted flowers. The genus *Rhododendron* has a high species richness in the Eastern Himalayas and southwestern China, which harbors more than 70% of all species in this genus on earth (Wang et al. 2018). Over the past 100 years, hundreds of *Rhododendron* species native to China have been introduced to Europe and the United States of America (USA), and thousands of varieties that have excellent ornamental characteristics and are easy to cultivate have been bred. In recent years, many rhododendron varieties are introduced to China from Europe and USA. Some of them perform well in cities and gardens in China, whereas some varieties have shown leaf burn in full light or flower poorly in low light. This indicates that their abilities to adapt to light intensity differ significantly. However, research on the photosynthetic adaptation of rhododendrons to growth irradiance is scarce (Song et al. 2019).

In the present study, the photosynthetic gas exchange, chlorophyll fluorescence and leaf water-related traits were investigated in two introduced varieties (*Rhododendron* 'XXL' and 'Furnivall's daughter') and one native species (*R. delavayi*) in *Rhododendron* grown under different growth irradiances. These experimental materials exhibit significantly different growth performances under high light. The main goals were to determine the differences in photosynthetic acclimation of rhododendrons to growth irradiance and the importance of leaf water-related traits on the acclimation of rhododendrons to growth irradiance. We predicted that: (1) there are significant differences in acclimation of photosynthesis to light intensity and leaf water-related traits among species or varieties; and (2) the plastic adjustment of leaf water-related traits will contribute to photosynthetic acclimation to different light intensities.

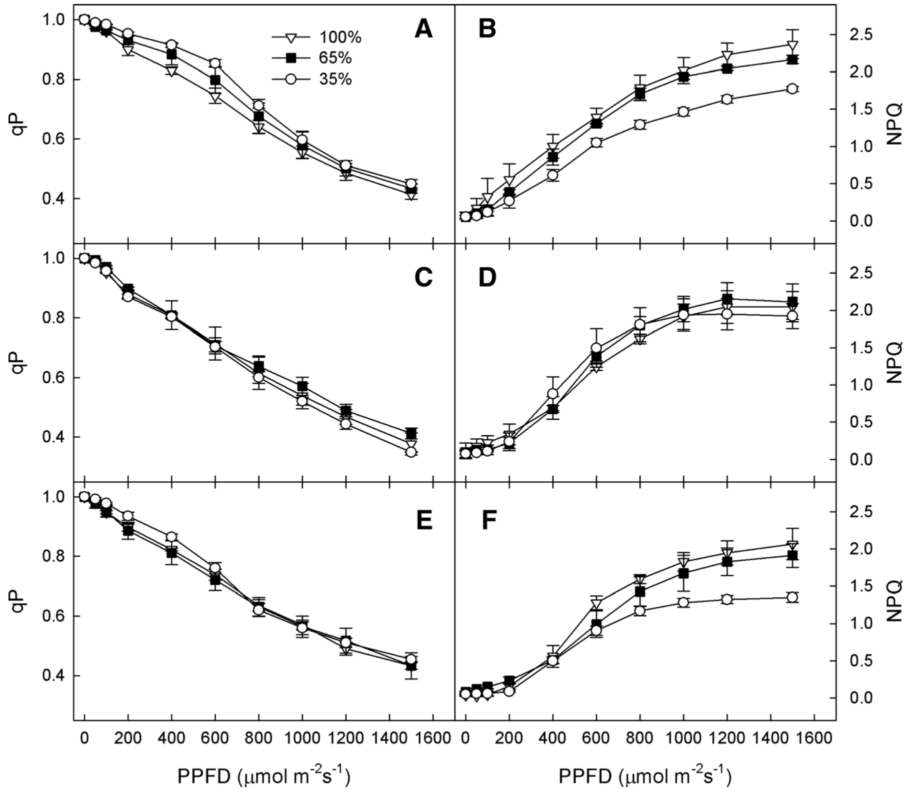

Fig. 2 Photochemical quenching coefficient (qP) and non-photochemical quenching (NPQ) of rhododendrons in response to photosynthetic photon flux density (PPFD) under different growth irradiances. **a**, **b** *Rhododendron* 'XXL'; **c**, **d** *Rhododendron* 'Furnivall's daughter'; **e**, **f** *Rhododendron delavayi*. Each point represents mean ± SE of 3 measurements from different plants

Materials and methods

Study site and plant materials

The study was conducted in Dachunhe Experimental Station (alt. 2050 m, 24° 34′ 45″ N, 102° 35′ 2″ E) of the Flower Research Institute of the Yunnan Academy of Agricultural Sciences in southwest China. The study site has a mean annual temperature of 14.8 °C, and an average annual precipitation of 900 mm, 80% of which occurs during the rainy season between May and October.

We compared photosynthetic adaptation to light intensity of two induced hybrids (*Rhododendron* 'Furnivall's daughter' and 'XXL') and one native species (*R. delavayi*). *Rhododendron* 'Furnivall's Daughter' was bred by a British breeder, Waterer (Knap Hill), in 1948, and is a potted plant with pink flowers. The parents of this variety are 'Mrs Furnival', which is an offspring of *R. griffithianum* and *R. caucasium*. *Rhododendron* 'XXL' was bred by Johan Vanderhaegen in Belgium, in 2001, and is a tetraploid variety with pink flowers (https://www.bundessortenamt.de/rhodo/index.cfm?job=rhodo_one&job2=herkunft&rid=47254). *R. delavayi* is an evergreen shrub or small tree with a height of 1–7 m. This species with large red flowers grows in mixed forests, forest margins, rocky slopes, and open fields at an altitude of 1200–3200 m in southwest China, and shows high tolerance to high light and drought stresses (Cai et al. 2015). These varieties or species were selected as experimental materials because they are widely cultivated in gardens, and have different growth performances in high light. Under low light, they flower significantly less than under high light. However, under full sunlight, even with adequate water supply, the leaves of *Rhododendron* 'Furnivall's daughter' are often burned, whereas leaf burn is not found in *Rhododendron* 'XXL' and *R. delavayi*.

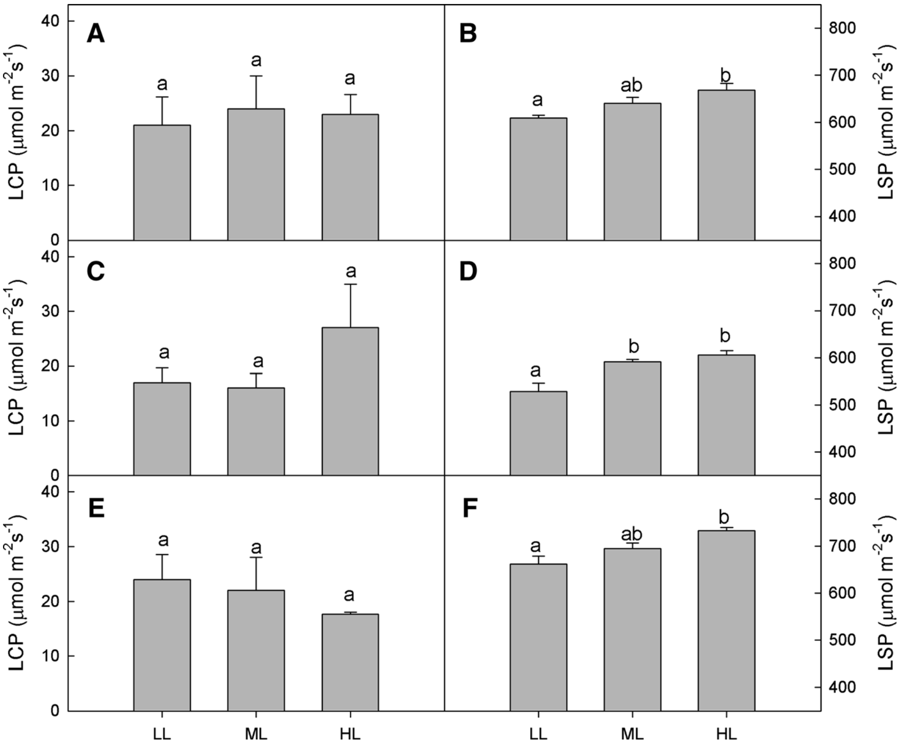

Fig. 3 Light compensation points (LCP) and light saturation points (LSP) of rhododendrons under 35% of full sunlight (LL), 65% of full sunlight (ML) and full sunlight (LL). **a**, **b** *Rhododendron* 'XXL'; **c**, **d** *Rhododendron* 'Furnivall's daughter'; **e**, **f** *Rhododendron delavayi*. Each data point represents the average of three independent measurements ± SE. Different letters above bars in each graph indicate statistically different mean values ($P < 0.05$), as determined by LSD multiple comparison tests

Two-year-old seedlings of *Rhododendron* 'Furnivall's Daughter' and 'XXL', and *R. dalavayi* were planted in plastic pots (30 cm in diameter) filled with peat moss. Before new leaves sprouted (March 21, 2017), the seedlings were exposed to full sunlight (high light, HL), medium light (ML, 65% of full sunlight), and low light (LL, 35% of full sunlight) with the use of shade nets. Thirty seedlings per light treatment for each species or each variety were planted. During treatment, the seedlings were given a slow release fertilizer (N: P: K, 15:15:15) every half month, and watered every 1–3 days as needed. From July 26 to August 2, 2017, newly formed, mature leaves were used for the measurements of photosynthesis, chlorophyll content, and anatomical traits. The seedlings of *R. delavayi* were obtained by seed propagation, while those of *Rhododendron* 'Furnivall's Daughter' and 'XXL' were cutting seedlings.

Photosynthetic measurements

Photosynthetic gas exchange and chlorophyll fluorescence in relation to light intensity were simultaneously measured from fully expanded leaves using a Li-Cor 6400 portable photosynthesis system with a 6400-40 fluorescence chamber (Lincoln, NE, USA). The values for minimum fluorescence and maximum fluorescence were measured before dawn. Before measurement, the leaf was illuminated by an actinic light of 1200 μmol m^{-2} s^{-1} (10% blue light, 90% red light) for 15 min to induce the maximum stomatal aperture. Photosynthetic light response curves of three leaves from different individuals per treatment of each variety or species were measured at 10 light levels between 1500 and 0 μmol photons m^{-2} s^{-1} under controlled levels of CO_2 (400 μmol mol^{-1}), flow rate (500 mmol s^{-1}), leaf temperature (25 °C) and leaf-to-air vapor pressure deficits (1.0–1.5 kPa) using an automated protocol built into Li-Cor 6400. The

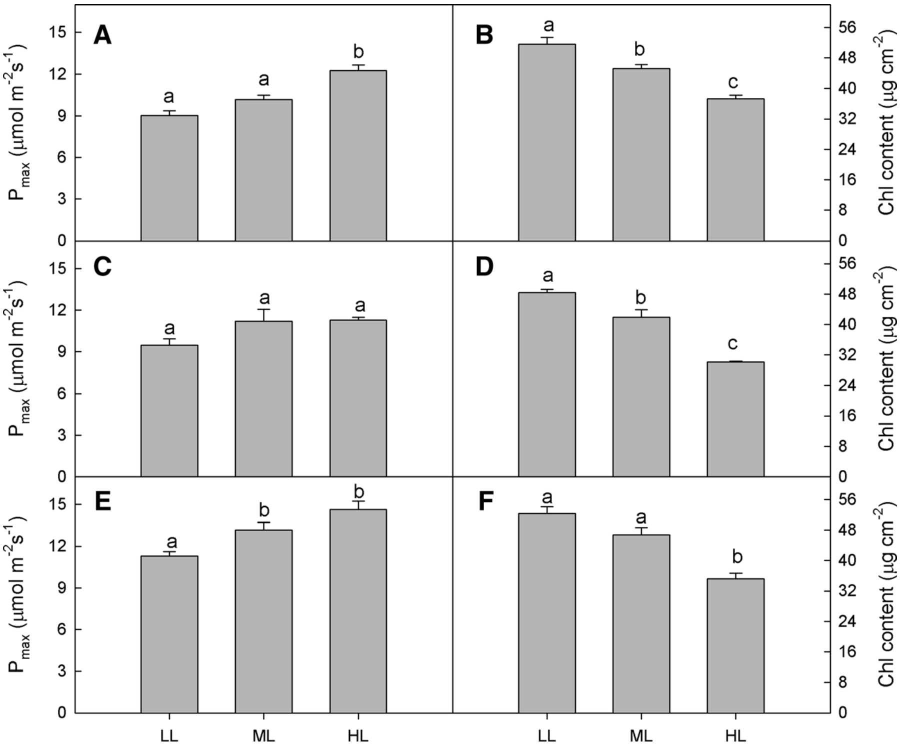

Fig. 4 Light-saturated photosynthetic rates (P_{max}) and chlorophyll (Chl) content per unit area of rhododendrons under 35% of full sunlight (LL), 65% of full sunlight (ML) and full sunlight (LL). **a**, **b** *Rhododendron* 'XXL'; **c**, **d** *Rhododendron* 'Furnivall's daughter'; **e**, **f** Rhododendron delavayi. Each data point represents the average of three independent samples ± SE. Different letters above bars in each graph indicate statistically different mean values ($P < 0.05$), as determined by LSD multiple comparison tests

program was configured to advance to the next step if the sum of the three coefficients of variation (CO_2, water vapor, and flow rate) was < 0.3%, with minimum wait time of 3 min. Each leaf was equilibrated to initial conditions by waiting at least 15 min before executing the automated protocol. Photosynthetic rate (P_n), stomatal conductance (g_s), transpiration rate (T_r), and fluorescence parameters were recorded during measurements. Values for light-saturated photosynthetic rate (P_{max}), light compensation point (LCP), and light saturation point (LSP) were fitted by a non-rectangular hyperbola (Prioul and Chartier 1977), while the electron transport rate of PSII (ETR), photochemical quenching (qP), and non-photochemical quenching (NPQ) were calculated according to the method of Genty et al. (1989).

Determination of leaf traits

To determine leaf area and leaf mass per unit area (LMA), we collected 10 mature leaves from 10 individuals per treatment for each variety or species. Leaf area was measured using a leaf area meter (LI-3000A, USA). Dry mass was determined after leaves were dried for 48 h at 70 °C. Then, LMA was calculated as leaf dry mass/area. Chlorophyll (Chl) was extracted in N,N-dimethylformamide for 48 h in the dark. Then, the absorbance was measured at 647.0 and 664.5 nm with a spectrophotometer (UV-2550, Shimadzu, Japan), and the chlorophyll content was calculated following the method of Inskeep and Bloom (1985).

To characterize stomatal traits, we collected six mature leaves per light treatment for each variety or species. Each leaf was divided along its midrib. One half of a leaf was soaked for 1 h in a 5% NaOH aqueous solution, while the other half was used for

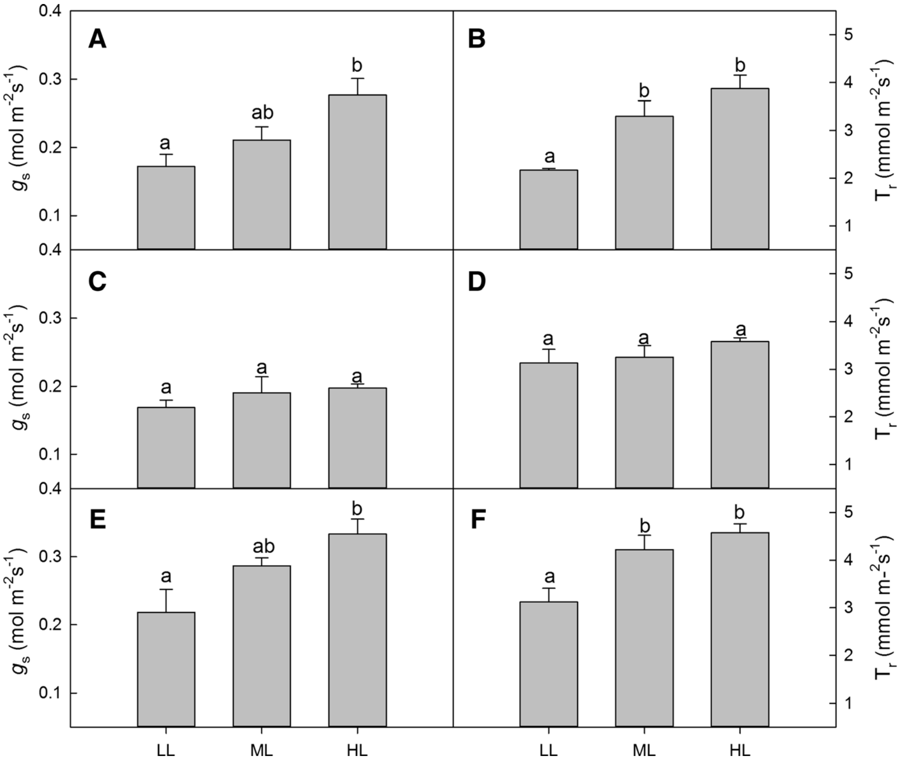

Fig. 5 Values for stomatal conductance (g_s) and transpiration rate (T_r) of rhododendrons under 35% of full sunlight (LL), 65% of full sunlight (ML) and full sunlight (LL). **a**, **b** *Rhododendron* 'XXL'; **c**, **d** *Rhododendron* 'Furnivall's daughter'; **e**, **f** *Rhododendron delavayi*. Each data point represents the average of three independent samples ± SE. Different letters above bars in each graph indicate statistically different mean values ($P < 0.05$), as determined by LSD multiple comparison tests

examination of the stomata. After leaves were cleaned, three sections were excised from the top, middle, and bottom portions, then stained with 1% safranin and mounted in glycerol to obtain the vein density (D_v). Samples were photographed at 10 × magnification with a digital camera mounted on a Leica DM2500 microscope (Leica Microsystems Vertrieb GmbH, Wetzlar, Germany). Vein lengths were determined from digital images via the IMAGEJ program (https://rsb.info.nih.gov/ij/). Values for D_v were expressed as vein length per unit area. For stomatal observations, the lower and upper epidermises were peeled from the middle portions of fresh leaves, and photographed under a Leica DM2500 microscope. Stomata were observed in 30 randomly selected fields. Stomatal density (SD) was calculated as the number of stomata per unit leaf area, and stomatal length (SL) represents the length of the guard cell.

Statistical analysis

To estimate the differences among light treatments for all parameters, we used one-way ANOVA and LSD multiple comparison tests. All statistical analyses were performed with SPSS 16.0 (SPSS Inc., Chicago, IL, USA).

Results

Photosynthetic performance and chlorophyll content under different growth irradiances

The photosynthetic rates (P_n) and PSII electron transport rates (ETR) of all plants at high light (HL), medium light (ML), and low light (LL) increased greatly with higher PFFDs (Fig. 1). Non-photochemical quenching (NPQ) also increased in these same plants and treatments in response to higher PPFDs, while all plants showed decreased photochemical

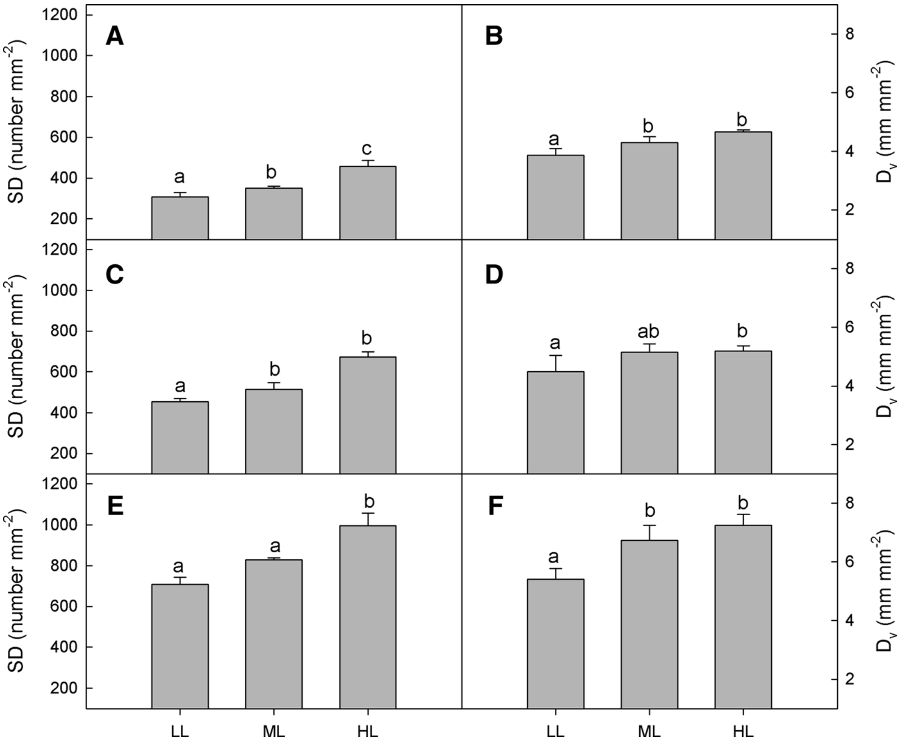

Fig. 6 Values for stomatal density (SD) and vein density (D_v) of rhododendrons under 35% of full sunlight (LL), 65% of full sunlight (ML) and full sunlight (LL). **a**, **b** *Rhododendron* 'XXL'; **c**, **d** *Rhododendron* 'Furnivall's daughter'; **e**, **f** *Rhododendron delavayi*. Each data point represents the average of ten independent samples ± SE. Different letters above bars in each graph indicate statistically different mean values ($P < 0.05$), as determined by LSD multiple comparison tests

quenching coefficients with increasing PPFDs (Fig. 2). P_n reached maximums at lower PPFD levels than did ETR and NPQ. For *Rhododendron* 'XXL' and *R. delavayi*, the NPQ values of plants grown at HL were higher than those of plants grown at ML and LL; however, no significant difference in NPQ was observed in *Rhododendron* 'Furnivall's daughter' plants at these irradiances.

There was no significant difference in light compensation point (LCP) among the three rhododendrons grown in HL, ML, or LL; however, light saturation points (LSP) in leaves of HL plants were higher than those of LL plants. At all light treatments, the LSP values in *Rhododendron* 'XXL' and *R. delavayi* were higher than those in *Rhododendron* 'Furnivall's daughter' (Fig. 3). The light-saturated photosynthetic rate (P_{max}) of *Rhododendron* 'XXL' and *R. delavayi* under HL conditions were higher than those under ML and LL conditions; however, the leaves of *Rhododendron* 'Furnivall's daughter' showed no significant difference in P_{max} among light treatments (Fig. 4). At all growth irradiances, the P_{max} in *Rhododendron* 'XXL' and *R. delavayi* were higher than those in *Rhododendron* 'Furnivall's daughter'. The values for stomatal conductance (g_s) and transpiration rate (T_r) increased with irradiance levels in *Rhododendron* 'XXL' and *R. delavayi*, while no significant differences in g_s and T_r values for *Rhododendron* 'Furnivall's daughter' were found among light treatments (Fig. 5). Chlorophyll contents of *Rhododendron* 'XXL' and 'Furnivall's daughter', and *R. delavayi* were significantly affected by growth irradiance (Fig. 4). Specifically, chlorophyll contents of all plants decreased with increasing light levels. In addition, in response to increasing light levels, the chlorophyll content of *Rhododendron* 'Furnivall's daughter' decreased more sharply than did those of *Rhododendron* 'XXL' and *R. delavayi*.

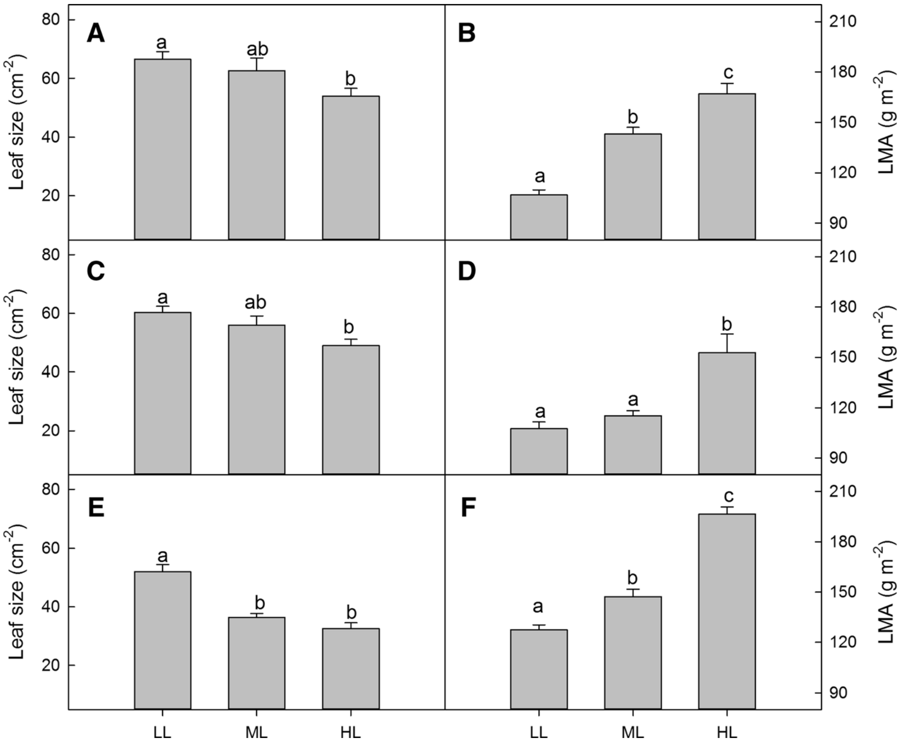

Fig. 7 Values for leaf size and leaf dry mass per unit area (LMA) of rhododendrons under 35% of full sunlight (LL), 65% of full sunlight (ML) and full sunlight (LL). **a**, **b** *Rhododendron* 'XXL'; **c**, **d** *Rhododendron* 'Furnivall's daughter'; **e**, **f** *Rhododendron delavayi*. Each data point represents the average of ten independent samples ± SE. Different letters above bars in each graph indicate statistically different mean values ($P < 0.05$), as determined by LSD multiple comparison tests

Leaf morphology and anatomy

Growth irradiance significantly affected leaf morphological and anatomical traits in three rhododendrons. When grown at higher light levels, stomatal density (SD) and vein density (D_v) increased in all plants (Fig. 6). Compared with the plants of *Rhododendron* 'XXL' and 'Furnivall's daughter', *R. delavayi* had higher values for SD and D_v, but smaller leaf size. Leaf dry mass per unit area of all plants increased when light levels increased, whereas leaf size decreased (Fig. 7).

Correlations among leaf traits

Photosynthetic rate and leaf water-related traits were positively correlated (Fig. 8). The P_{max} was positively correlated with g_s, SD, and D_v, but was not correlated with chlorophyll content. Meanwhile, both SD and D_v were positively correlated with g_s and T_r (Fig. 9). A positive correlation was observed between D_v and LSP, whereas D_v was negatively correlated with leaf size (Fig. 10). These results indicate that photosynthetic gas exchange and stomatal conductance are regulated by leaf water-related traits in rhododendrons grown under different light intensities.

Discussion

This study shows that the photosynthetic acclimation of rhododendron varieties and native species differs across growth irradiance levels, and that this photosynthetic acclimation to growth irradiance is related to leaf water-related traits. The value for P_{max} in the native species was higher than those of the two introduced varieties. We found that the native species had higher values for SD, D_v, and g_s than the two introduced varieties (Figs. 5, 6), and these three parameters were positively correlated with P_{max} (Fig. 8). However, the P_{max} of the native species and *Rhododendron* 'XXL' under high growth irradiance

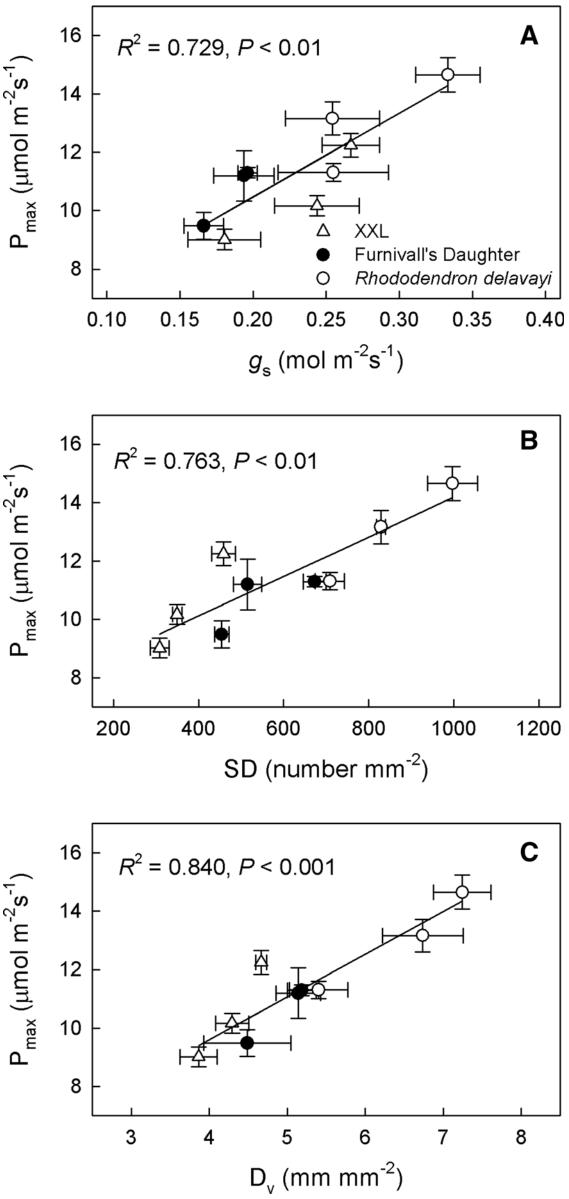

Fig. 8 Relationships between light-saturated photosynthesis (P_{max}) and **a** maximal stomatal conductance (g_s), and **b** stomatal density (SD) and **c** vein density (D_v) under different growth irradiances. open triangle, *Rhododendron* 'XXL'; filled circle, *Rhododendron* 'Furnivall's daughter'; open circle, *Rhododendron delavayi*

were higher than those under low growth irradiance; the P_{max} of *Rhododendron* 'Furnivall's daughter' remained unchanged under different irradiance levels. The light saturation points of the native species and *Rhododendron* 'XXL' under high growth irradiance were also higher than those of *Rhododendron* 'Furnivall's daughter'. These findings are consistent with our observations in the field that the leaves of *Rhododendron* 'Furnivall's daughter' are often burned under full sunlight, whereas leaf burn is not found in *Rhododendron* 'XXL' or *R. delavayi*. Taken together, these findings indicate that the native species and *Rhododendron* 'XXL' have higher photosynthetic plasticity to light intensity, and higher tolerance to high light than *Rhododendron* 'Furnivall's daughter'. Previous studies have suggested that the photosynthetic acclimation of plants to growth irradiance can be linked to leaf morphology and anatomy, the balance of light absorption capacity of two photosystems, and xanthophyll cycle pigments (Rozendaal et al. 2006; Hallik et al. 2011; Zhang et al. 2017). These traits enable plants to deal with excessive energy under high light conditions or to increase light absorption under low light conditions (Lai et al. 2005). Here, we found that the plasticity in leaf water-related trait provided an efficient means of regulating photosynthetic acclimation of rhododendrons to growth irradiance.

Leaf sizes of the tested plants increased when light levels increased, but transpiration rate increased. When plants are exposed to high light, the temperatures of their leaves may increase (Zhang et al. 2005; Barman et al. 2008). To avoid overheating and leaf burns, plants may increase transpiration to lower leaf temperatures. A large leaf can increase the capture of light energy, but it also increases the heat load and water demand. In contrast, a small leaf is more adaptable to high irradiance conditions because it has a lower transpirational cost than large leaf (Vogel 2009; Li et al. 2013). Thus, the plants exposed to high light may decrease their leaf sizes, and increase transpiration rates (Dai et al. 2009; Sack and Scoffoni 2013).

Leaf venation network must be adequate to supply enough water for a given stomatal pore area and given evaporative demand, because the quick replenishment of water lost due to transpiration is essential for the maintenance of normal physiological functions of the leaves (Brodribb and Jordan 2011; Scoffoni et al. 2011). Usually, the sun leaves of many species develop a higher vein density and stomatal density than shade leaves (Brodribb and Jordan 2011; Scoffoni et al. 2015). Vein density shows a negative correlation with species shade tolerance index (Sack and Scoffoni 2013). However, in some species, the vein densities of the leaves under high light conditions are not significantly different than those under low light conditions

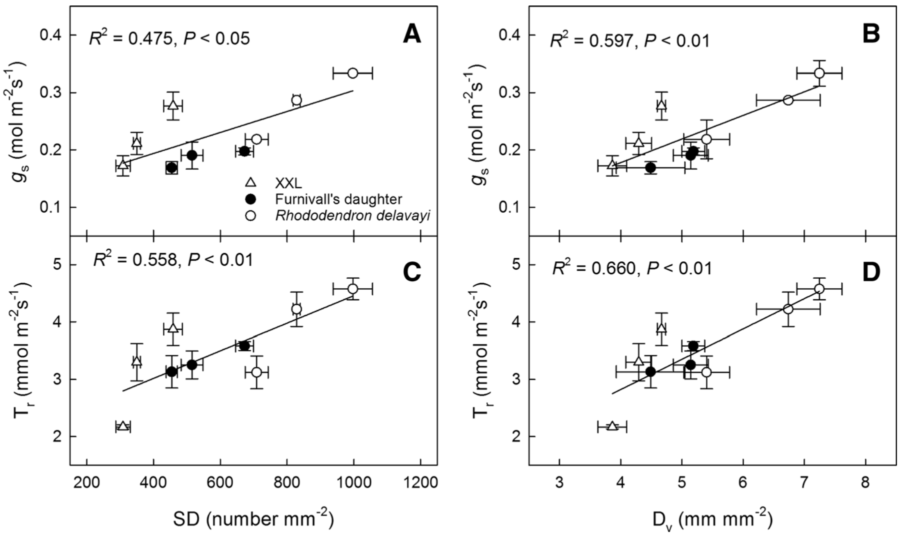

Fig. 9 Relationships between stomatal conductance (g_s) and stomatal density (SD), and vein density (D_v), and between transpiration rate (T_r) and SD, and D_v of three rhododendrons under different growth irradiances. open triangle, *Rhododendron* 'XXL'; filled circle, *Rhododendron* 'Furnivall's daughter'; open circle, *Rhododendron delavayi*

(Amiard et al. 2005). Vein and stomatal densities are regulated by differential leaf expansion such that leaf hydraulic conductance and stomatal conductance remain proportional; thus, leaf size plasticity provides an efficient way for plants to acclimate hydraulic conductance and stomatal conductance to the contrasting evaporative conditions of sun and shade (Murphy et al. 2012). Low vein density may reduce construction cost and lower vein projected area, and potentially enhance light capture in shade (Sack and Scoffoni 2013). The most efficient investment in vein and stomata occurs when the supply of water to evaporative surfaces induces the full openness of stomata under saturating light conditions (Murphy et al. 2014). Thus, a suit of physiological, stomatal, and anatomical traits tends to shift together during the adaptation to diverse light regimes (Brodribb and Jordan 2011; Murphy et al. 2012; Scoffoni et al. 2015). We found that SD and D_v increased with greater growth irradiance in rhododendrons, and were correlated positively with g_s, T_r, and P_{max}. A positive correlation was also found between D_v and light saturation point. These indicate that enough water supply may improve leaf water status to support the increased transpirational demand and prevent stomatal closure, thereby improving light energy utilization efficiency under high light conditions. Previous studies have suggested that leaf photosynthetic rate is related to water transport capacity (Sack and Frole 2006; Brodribb et al. 2007). However, the negative correlation between vein density and leaf size indicates that the vein investment per unit leaf area is lower under lower irradiance than higher irradiance. Thus, the adjustments of vein density and stomatal density play important roles in the photosynthetic acclimation of rhododendrons to growth irradiances.

Sunlight is one of the major environmental factors that influence photosynthesis and growth, but excess light may damage photosynthetic apparatus, and cause depression of photosynthetic efficiency (Kato et al. 2003; Aleric and Kirkman 2005). The main responses to low and high irradiance levels are to increase light capture and energy dissipation, respectively, which are reflected by changes of chlorophyll content and chlorophyll fluorescence (Xu et al. 2009). Here, we found that chlorophyll contents of all plants decreased in response to increasing irradiance levels. This implies that greater fractional nitrogen investments in chlorophyll and light-harvesting pigment-binding complexes under low irradiance (Hikosaka and Terashima 1995). Previous studies have suggested that strong irradiance may destroy chlorophyll, and plants can optimize the efficiency of light absorption by decreasing chlorophyll content per unit leaf area (Wittmann et al. 2001; Jason et al. 2004). The reduction of chlorophyll content may

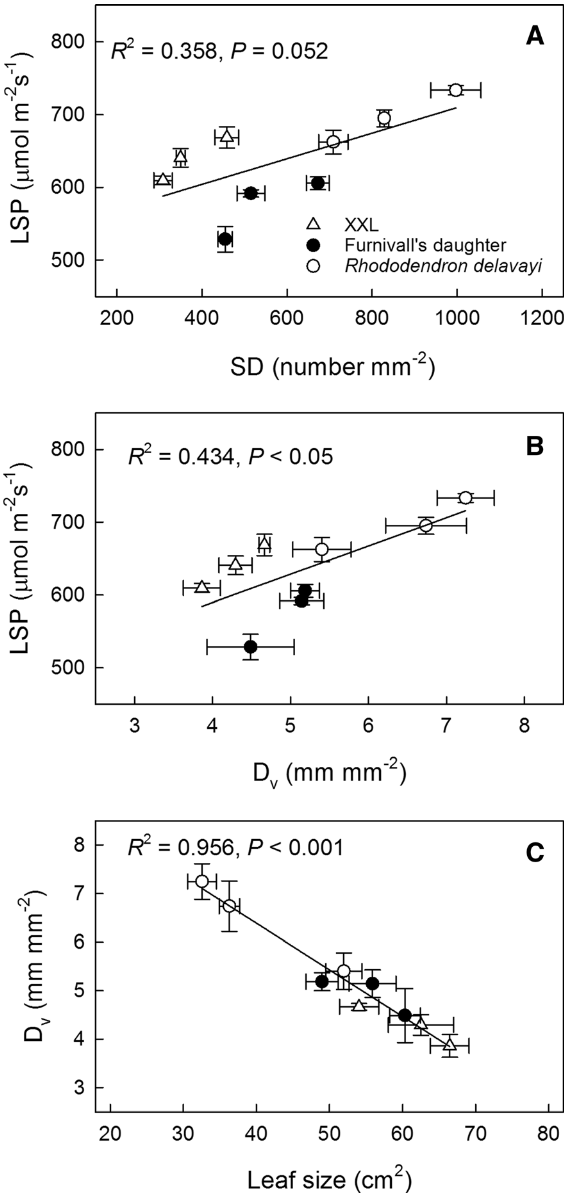

Fig. 10 Relationships between light saturation point (LSP) and stomatal density (SD), and vein density (D_v), and between D_v and leaf size in three rhododendrons under different growth irradiances. open triangle, *Rhododendron* 'XXL'; filled circle, *Rhododendron* 'Furnivall's daughter'; open circle, *Rhododendron delavayi*

decrease photosynthetic capacity (Mao et al. 2007; Dai et al. 2009). However, the values for P_{max} of rhododendrons were not correlated with chlorophyll content per unit leaf area. Thus, the marked decrease in chlorophyll content under high irradiance may be a strategy for rhododendrons to reduce light absorption to avoid photodamage.

High photochemical quenching (qP) is advantageous for the separation of electric charge in the reaction center and PSII yield (Mao et al. 2007). Here, we found that the excess light energy in the leaves of rhododendrons increased with increasing irradiance levels. *D*1 protein is a target of high light-induced damage to the PSII complex, and its turnover is accelerated when irradiance increases (Aro et al. 1993). The relaxation of non-photochemical quenching (NPQ) is an important strategy for avoiding the deleterious effects of excess light energy (Müller et al. 2001). Higher value of NPQ reflects a greater degree of thermal dissipation (Germino and Smith 2001). Under high light, NPQ can be enhanced to protect the photosynthetic apparatus from photoinhibition by enriching xanthophyll cycle pigments (Golding and Johnson 2003; Ballottari et al. 2007). Here, we found that the NPQ values of *Rhododendron* 'XXL' and *R. delavayi* plants under high growth irradiance were higher than those of plants under medium and low irradiances, while no significant difference in NPQ was observed in *Rhododendron* 'Furnivall's daughter' at these irradiances. This indicates that low activation of NPQ may contribute to low tolerance of *Rhododendron* 'Furnivall's daughter' plants to high irradiance.

In conclusion, our study revealed substantial differences in photosynthetic acclimation to light intensity among rhododendrons. The native species *R. delavayi* and *Rhododendron* 'XXL' had higher photosynthetic plasticity to light intensity, and higher tolerance to high light than *Rhododendron* 'Furnivall's daughter'. Observed increases in vein density may increase water supply to the leaves to meet transpirational demand under high light condition. In addition, the decrease in chlorophyll content and the increase in non-photochemical quenching observed when plants are exposed to high light may avoid photodamage of PSII by decreasing leaf absorption and enhancing heat dissipation. These results suggest that increasing water supply to the leaves and reducing the photodamage of PSII play important roles in the photosynthetic acclimation of rhododendrons to growth irradiance, and the poor tolerance of *Rhododendron* 'Furnivall's daughter' to high light may be due to its low capacities for water supply and heat dissipation. Our findings may contribute to the development of cultivation strategy and the understanding of ecological adaptation in rhododendrons.

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