

## GROWTH, PHOTOSYNTHETIC AND BIOCHEMICAL RESPONSES OF RHODODENDRON DELAVAYI FRANCH SEEDLINGS IRRIGATED WITH DIFFERENT pH SOLUTIONS

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### Abstract

*Rhododendron delavayi* Franch, an evergreen ornamental shrub, has a special preference for acidic soils, however, the exact pH value of the matrix suitable for *R. delavayi* growth is still unknown. In this study, three-year-old *R. delavayi* seedlings were irrigated with different pH solutions (4, 4.5, 5, 5.5, 6, 6.5, 7, and groundwater) to observe their growth, photosynthetic, and biochemical responses. Results showed that *R. delavayi* seedlings maintained well-growth and leaf development under pH 5.5 solution irrigation, without a remarkable difference in the branch numbers per plant. Meanwhile, the maximum values of the chlorophyll content, photosynthesis ability, and antioxidant enzyme activities were observed. Redundancy analysis indicated that the higher net photosynthesis rate and total chlorophyll content of *R. delavayi* seedlings were contributed to the rapid growth and improvement resistance of individuals. Our study suggested that the irrigation under pH 5.5 solution was the optimal treatment for the cultivation of *R. delavayi* seedling. The study provides a reliable scientific basis for exploring efficient cultivation and adaptation of *R. delavayi* seedlings under different pH matrixes.

**Key words:** *Rhododendron delavayi* Franch, Acidic soil, Growth, Photosynthesis, Antioxidant enzyme, Redundancy analysis.

### Introduction

Soil pH plays a vital role in regulating the soil physicochemical property and nutrient supply capacity, thereby affecting plant growth and stress resistance (Neina, 2019; Li *et al.*, 2020; Panico *et al.*, 2020). Acid soil is characterized by high hydrogen ion concentration. More hydrogen ions make the soil stiff and inhibit plant growth (Kozłowski, 1999). Meanwhile, iron and aluminum become more active in soil, which often form iron and aluminum phosphate salts with low solubility (Huang *et al.*, 2016), these salts are so difficult to absorb by plants that capacity of soil fertility supply is reduced. Thus, heavily acidic soil causes a series of problems, such as destroying the natural structure of soil, reducing nutrient availability, and producing excess organic acids (Lauricella *et al.*, 2020; Da Silva *et al.*, 2021; Jayme *et al.*, 2021). In these cases, plants cannot easily obtain nutrient elements and water from the soil, and the physiological metabolism of individuals is inhibited (Atta *et al.*, 2020). Therefore, studying the adaptability of plant growth to the degree of soil acidity is greatly important.

Generally, most plants have special preference and adaptability to the acidity and alkalinity of the matrix to survive (Chen 2018; Gupta *et al.*, 2020). When the pH ranges exceed the certain threshold that plant can tolerate, the physiological activities of plants, such as chlorophyll (Chl) content, net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), intercellular carbon dioxide ( $CO_2$ ) concentration ( $C_i$ ), and transpiration rate ( $E$ ), are affected and causing stress or death (Qi *et al.*, 2018). For instance, physiological responses, such as low growth rate, decreased  $P_N$ , disordered metabolic mechanisms indicated by the activated antioxidant enzymes, and increasing proline (Pro) content, could be caused by extremely low pH in soil. Previous research has shown that the soil acidification can reduce plant diversity and alter the composition of forest communities in forest ecosystems (Zarfos *et al.*, 2019).

Meanwhile, a study has found that the growth and development of blueberry fruit are very suitable under the pH 4.0-5.0 soil (Kim *et al.*, 2009). Therefore, plant species' acid-base adaptability to the substrate is important to ensure the normal growth of plants.

*Rhododendron delavayi* Franch, Ericaceae, is an evergreen woody ornamental plant of Yunnan-Guizhou Plateau, China (Zhang *et al.*, 2017; Ma *et al.*, 2010). Its brilliant red flowers have great ornamental value and can also be used to extract natural pigments (Li *et al.*, 2020). In addition, it is an important original breeding parent because of its cold environmental adaptability. At present, *R. delavayi* has been widely used in landscaping, home gardening, forest parks, and scenic spots and has a large market demand (Sharma *et al.*, 2014). Thus far, scholars have conducted substantial research on *R. delavayi*, such as rapid propagation techniques (Bu *et al.*, 2020), drought resistance abilities (Cai *et al.*, 2015), draft genome assembly (Li *et al.*, 2020), and liking acid soil (Fu *et al.*, 2019). Although this work has greatly strengthened people's understanding of the survival characteristics of *R. delavayi*, the exact pH value of the matrix suitable for *R. delavayi* growth is still unknown. Thus, the efficiency of artificial breeding of *R. delavayi* seedlings is greatly limited. The present work focuses on (1) the responses of growth, photosynthesis, biochemical parameters of *R. delavayi* seedlings under different pH solutions of irrigation; (2) the relationships among growth, development, and biochemical characteristics of *R. delavayi* seedlings. The study provides a reliable scientific basis for exploring efficient cultivation and adaptation of *R. delavayi* seedlings under different pH matrixes.

### Materials and Methods

**Experimental site and plant materials:** The experiments were carried out at an experimental farm, Guizhou University (N 26°27', E 106°39', altitude 1142 m), which

is located at the central region of Guizhou Province, Southwest China. A total of 100 three-year-old *R. delavayi*, well-grown and almost of similar size seedlings, obtained from a common garden nearby the experimental farm, were planted in plastic plots (24 cm in height × 32 cm in diameter). Each seedling was planted in the pot central, and then filled with 5 kg of acid yellow loam with pH value of 5 in April 10, 2019. All plants were grown in a controlled glasshouse (natural sunlight, temperatures of 18°C-25°C, relative humidity of 75%-80%) at the experimental farm, and each pot was watered 1 L of groundwater (pH = 6.8, Ca 71.5 mg L<sup>-1</sup>, Mg 17.2 mg L<sup>-1</sup>) every two days to ensure soil moisture. Forty healthy and uniform sized plants [height (H) 13.0 ± 1.0 cm, stem base diameter (BD) 0.15 ± 0.05 cm] were selected and equally divided into eight groups. Seven of them were irrigated with different pH solutions (4, 4.5, 5, 5.5, 6, 6.5, and 7), and one group served as a control check (CK) irrigated with groundwater. pH solutions in the amount of 1 L were adjusted to eight gradient values (T<sub>4</sub>, T<sub>4.5</sub>, T<sub>5</sub>, T<sub>5.5</sub>, T<sub>6</sub>, T<sub>6.5</sub>, and T<sub>7</sub>) with 98.3% H<sub>2</sub>SO<sub>4</sub> and NaOH to water the seedlings every two days, and CK was still irrigated with 1 L groundwater at the same time. The treatment started on June 1, 2019. After continuous treatment for two months (62 d), we began to measure the growth and physiological indexes of all plants.

**Soil pH:** Soil pH was measured at the end of the treatments. Soil was sampled at 10-20 cm depth in each pot to determine the pH values with the pH-meter (PHSJ-6L, LEICI Inc., Shanghai, China).

**Growth and leaf development of seedlings:** At the end of the experiment, the BD and H of each seedling, the branch numbers (BN), and leaf numbers (LN) per plant were determined. The leaf area (LA) per plant was measured by an AM350 (ADC Bio-Scientific Ltd, Herts, UK).

**Chl content:** The third to fifth whole fresh leaf on the branch were gathered and cut into pieces. The 100 mg samples of the clipped leaves were placed into 10 mL leaching solution (80% acetone) for more than 24 h, shaking until the leaf tissue turned white. The obtained extract was used to determine the absorbance at 663.6 and 646.6 nm using Spectrophotometer (UV-2000, Shimadzu, Kyoto, Japan). The contents of chlorophyll *a* (Chl *a*) and chlorophyll *b* (Chl *b*) were calculated by the optimized equations (Porra, 2002):

$$\begin{aligned} \text{Chl } a &= 13.71 A_{663.6} - 2.85 A_{646.6} \\ \text{Chl } b &= 22.39 A_{646.6} - 5.42 A_{663.6} \end{aligned}$$

Finally, the total Chl content [Chl (*a+b*)] and Chl (*a/b*) was calculated.

**Photosynthesis parameter measurements:** Light-response curves were determined using the portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA) with a red-blue light source (6400-02B, LI-

COR Inc., Lincoln, NE, USA). During the course of measurements, CO<sub>2</sub> concentration (C<sub>a</sub>), relative air humidity, and leaf temperature in the chamber were maintained at 380 ± 5 μmol mol<sup>-1</sup>, 75%, and 25°C, respectively. Photon flux density (PPFD) was set at 12 levels, decreasing from 1,800 μmol m<sup>-2</sup> s<sup>-1</sup> to 0 μmol m<sup>-2</sup> s<sup>-1</sup> at varying intervals in order to better fit the PPFD-P<sub>N</sub> response curve. Simultaneously, photosynthetic parameters were determined using the same instrument maintaining the same CO<sub>2</sub> concentration, relative air humidity, and leaf temperature in the chamber with PPFD at 1,200 μmol m<sup>-2</sup> s<sup>-1</sup>. For example, P<sub>N</sub>, g<sub>s</sub>, C<sub>i</sub>, and E were automatically recorded by the instrument.

Fresh leaves from the treatment and CK seedlings were selected for use in the measurements. All the measurement works were performed between 9:30 and 11:00 h under the clear days from August 1 to 5, 2019.

**Antioxidant enzymes and Pro:** Antioxidant enzyme activities, such as superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), and Pro content were determined using the following method (Nakama & Asada 1981). Each 0.5 g of powdered leaf was homogenized with an extraction buffer containing 1 mM ethylene diamine tetraacetic acid (EDTA), 50 mM phosphate buffer (pH 7.2), 1 g polyvinyl pyrrolidone (PVP), and 0.5% (v/v) Triton X-100. The homogenate was centrifuged (under 12,000 × g for 20 min at 4°C), and the supernatant was used to analyze the enzyme activities.

SOD activity was determined by the photochemical reduction of NBT (nitroblue tetrazolium), according to the ability to cause 50% inhibition of NBT reduction at 560 nm (Becana *et al.*, 1986). POD activity was measured by the guaiacol oxidation method (Nakano & Asada, 1981), and its activity was determined by observing the increase in absorbance at 470 nm for 1 min because of guaiacol oxidation. Meanwhile, CAT activity was elucidated using the method of Chance *et al.*, (1979) by monitoring the disappearance of H<sub>2</sub>O<sub>2</sub> at 240 nm at 25°C, amount of which was determined by the decrease at 240 nm for 1 min owing to H<sub>2</sub>O<sub>2</sub> consumption.

Pro content was determined by the method described by Bates *et al.*, (1973). Fresh leaf samples were homogenized with 2 mL of 40% ethanol in a cold sterile mortar. After agitation for 10 min, the extract was filtered through filter paper, and a 2-mL aliquot was used. Absorbance was measured in spectrophotometer (UV-2000, Shimadzu, Kyoto, Japan) at 528 nm. Pro content of fresh leaves was calculated by the proline standard curve.

**Data processing:** The value of water use efficiency (*WUE*) was calculated as P<sub>N</sub>/E (Guo *et al.*, 2019), and stomatal limitation (*L<sub>s</sub>*) was calculated as 1 - C<sub>i</sub>/C<sub>a</sub>, where C<sub>a</sub> was the ambient CO<sub>2</sub> concentration (Li *et al.*, 2013).

Nonrectangular hyperbola model (Farquhar & Von Caemmerer 1982) was used to fit the PPFD-P<sub>N</sub> response curves of plant leaves by SPSS 20.0 (SPSS, Chicago, USA). This equation model is as follows:

$$P_N = \frac{\alpha \text{PPFD} + P_{N\max} \pm \sqrt{(\alpha \text{PPFD} + P_{N\max})^2 - 4\alpha \text{PPFD} P_{N\max}}}{2K} - R_d$$

where  $K$  and  $P_{Nmax}$  are the convexity of the light response curve and the maximum net photosynthesis rate, respectively.  $\alpha$  is the apparent quantum yield and equal to the initial slope of the linear regression between  $P_N$  and PPFD below  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , and  $R_d$  is the dark respiration rate. Light compensation point ( $L_{CP}$ ) and light saturation point ( $L_{SP}$ ) were calculated as the PPFD at  $P_N$  equal to zero and  $P_{Nmax}$  for each curve, respectively.

All values were the means  $\pm$  SD of five replicate seedlings. Data were analyzed using SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was analyzed to determine the effects of pH on the growth and development of seedlings and leaf photosynthesis parameters by the Fisher's LSD test. Redundancy analysis (RDA) was used to assess the relationships among growth and development of seedlings, Chl content, photosynthetic, and biochemical parameters by OriginPro 2020 (Microcal Inc., Northampton, MA, USA); it was also used to plot the figures.

## Results

**Soil pH:** At the end of the experiment, soil pH was similar to the pH of irrigation solutions (Table 1), suggesting that soil acid in each pot was significantly influenced by the different pH solutions in addition to  $T_5$  treatment. Compared with the pH of irrigating solutions, soil pH values in  $T_4$  and  $T_{4.5}$  were higher and lower in  $T_{5.5}$ ,  $T_6$ ,  $T_{6.5}$ , and  $T_7$ . Meanwhile, soil acidity in  $T_5$  indicated no significant change.

**Seedling growth and leaf development:** The parameters, H, BD, BN, LN, and LA, of *R. delavayi* seedlings were analyzed (Table 2). Compared with CK, the values of BD and H of seedlings under  $T_{5.5}$  and  $T_6$  were higher without significant difference in  $T_{4.5}$  and  $T_5$ . The minimum values of H and BN were observed in  $T_4$ . However, no significant difference in BN per plant was found between CK and the treatments. The maximum and minimum values of LN appeared in  $T_{5.5}$  and  $T_{4.5}$ . The LN per plant also had no significant difference in  $T_4$  and  $T_{4.5}$ . The LA per plant of *R. delavayi* seedlings was larger in CK,  $T_5$ ,  $T_{5.5}$ , and  $T_6$ . These findings indicated that, apart from the branch numbers per plant, the irrigation solutions with different pH values had significant impact on the growth and leaf development of *R. delavayi* seedlings.

**Chl content:** Fig. 1 reflects the change trend of Chl *a*, Chl *b*, and total Chl contents and Chl (*a/b*) of *R. delavayi* seedlings irrigated with the different pH solutions. The highest contents of Chl *a*, Chl *b*, and total Chl of leaves appeared in  $T_{5.5}$ , which were 2.54, 0.72, and 3.26  $\text{mg g}^{-1}$ , respectively (Fig. 1). The contents of Chl *a*, Chl *b*, and total Chl continued to increase along the gradient of  $T_4$ ,  $T_{4.5}$ ,  $T_5$ , and  $T_{5.5}$ , and then decreased from  $T_6$ ,  $T_{6.5}$ , to  $T_7$ . In the meantime, the values of Chl (*a/b*) increased from  $T_4$  to  $T_6$ , reached the maximum in  $T_{6.5}$ , and then finally decreased in  $T_7$ .

**PPFD- $P_N$  response curves and fitting parameters:** The PPFD- $P_N$  response curves indicated the ability of plants to assimilate external light energy. As shown in (Fig. 2), the PPFD- $P_N$  response curves of *R. delavayi* seedlings in CK,  $T_5$ ,  $T_{5.5}$ , and  $T_6$ , were relevantly close to each other. Meanwhile, we observed that when the PPFD was from  $0 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ ,  $P_N$  values increased almost linearly with increasing PPFD. When PPFD was greater than  $200 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the growth rate of  $P_N$  became slow. Then,  $P_N$  curves gradually flattened with increasing PPFD until light intensity saturated at one point.  $P_N$  values remained unchanged or decreased slightly when the PPFD ranged from  $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$  to  $1800 \mu\text{mol m}^{-2} \text{s}^{-1}$ .

The nonrectangular hyperbola model was used to fit the PPFD -  $P_N$  response curves to obtain the parameters for *R. delavayi* seedlings under different pH solutions of irrigation (Table 3).  $P_{Nmax}$  characterizes the maximum value of plant photosynthetic potential, and  $\alpha$  reflects the conversion and utilization efficiency of light energy at the weak light stage. According to Table 3, the values of  $P_{Nmax}$  in CK,  $T_5$ ,  $T_{5.5}$ , and  $T_6$  were significantly higher than all other treatments, and they had no significant differences. In the meantime, the minimum  $P_{Nmax}$  appeared in  $T_4$ . The higher values of  $L_{CP}$  were observed in  $T_4$  and  $T_{4.5}$  and the minimum in  $T_{5.5}$ . In addition, the lowest value of  $L_{SP}$  was observed in  $T_{5.5}$ , and its maximum appeared in  $T_6$ . The values of  $R_d$  in  $T_{6.5}$  and  $T_7$  had no significant differences and were significantly higher than all other treatments. In all treatments, *R. delavayi* seedlings had higher values of  $\alpha$  in  $T_5$  and  $T_{5.5}$  and lower values in  $T_4$  and  $T_{4.5}$ .

Table 1. Soil pH in each pot irrigated with different pH solutions.

Treatments	CK	$T_4$	$T_{4.5}$	$T_5$	$T_{5.5}$	$T_6$	$T_{6.5}$	$T_7$
Soil pH	5.21 $\pm$ 0.20 <sup>c</sup>	4.23 $\pm$ 0.15 <sup>g</sup>	4.56 $\pm$ 0.08 <sup>f</sup>	5.04 $\pm$ 0.17 <sup>c</sup>	5.36 $\pm$ 0.21 <sup>d</sup>	5.89 $\pm$ 0.09 <sup>c</sup>	6.41 $\pm$ 0.16 <sup>b</sup>	6.85 $\pm$ 0.23 <sup>a</sup>

Table 2. Effects of irrigation with different pH solutions on the growth and morphology of *R. delavayi* seedlings.

Treatments	BD (cm)	H (cm)	BN	LN	LA per plant (cm <sup>2</sup> )
CK	0.38 $\pm$ 0.10 <sup>b</sup>	19.23 $\pm$ 1.04 <sup>b</sup>	1.92 $\pm$ 0.54 <sup>a</sup>	20.17 $\pm$ 2.04 <sup>c</sup>	152.34 $\pm$ 32.02 <sup>a</sup>
$T_4$	0.34 $\pm$ 0.08 <sup>c</sup>	16.02 $\pm$ 0.84 <sup>c</sup>	2.03 $\pm$ 0.32 <sup>a</sup>	19.24 $\pm$ 3.12 <sup>d</sup>	136.25 $\pm$ 27.12 <sup>b</sup>
$T_{4.5}$	0.40 $\pm$ 0.07 <sup>b</sup>	18.84 $\pm$ 1.33 <sup>b</sup>	2.02 $\pm$ 0.21 <sup>a</sup>	19.08 $\pm$ 2.95 <sup>d</sup>	132.67 $\pm$ 15.06 <sup>b</sup>
$T_5$	0.39 $\pm$ 0.10 <sup>b</sup>	21.51 $\pm$ 1.27 <sup>b</sup>	2.02 $\pm$ 0.05 <sup>a</sup>	20.30 $\pm$ 3.02 <sup>c</sup>	151.73 $\pm$ 25.17 <sup>a</sup>
$T_{5.5}$	0.44 $\pm$ 0.08 <sup>a</sup>	27.72 $\pm$ 1.54 <sup>a</sup>	2.03 $\pm$ 0.37 <sup>a</sup>	27.11 $\pm$ 2.64 <sup>a</sup>	154.23 $\pm$ 18.03 <sup>a</sup>
$T_6$	0.42 $\pm$ 0.09 <sup>a</sup>	26.08 $\pm$ 0.93 <sup>a</sup>	2.11 $\pm$ 0.53 <sup>a</sup>	24.56 $\pm$ 1.96 <sup>b</sup>	158.65 $\pm$ 22.17 <sup>a</sup>
$T_{6.5}$	0.37 $\pm$ 0.07 <sup>b</sup>	18.64 $\pm$ 1.10 <sup>b</sup>	1.98 $\pm$ 0.36 <sup>a</sup>	19.82 $\pm$ 2.84 <sup>c</sup>	139.22 $\pm$ 19.04 <sup>b</sup>
$T_7$	0.39 $\pm$ 0.06 <sup>b</sup>	19.03 $\pm$ 0.89 <sup>b</sup>	2.04 $\pm$ 0.21 <sup>a</sup>	21.64 $\pm$ 3.24 <sup>c</sup>	125.78 $\pm$ 21.06 <sup>c</sup>

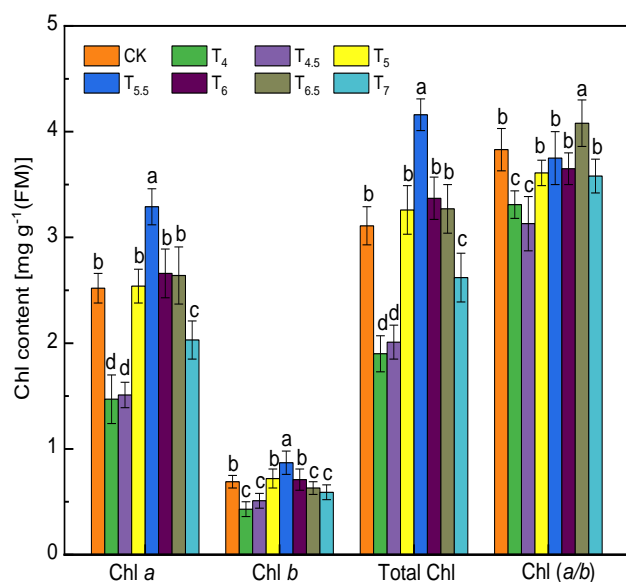


Fig. 1. Contents of Chl *a*, Chl *b*, and total Chl and Chl (*a/b*) of *R. delavayi* leaves under different pH solutions of irrigation.

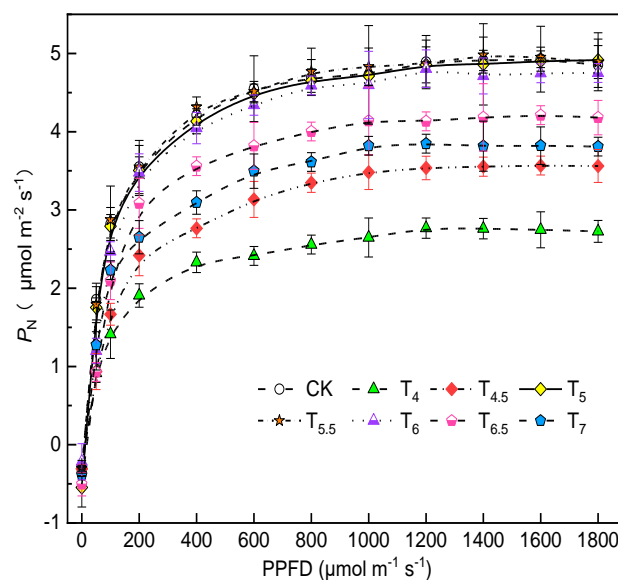


Fig. 2. PPF (photon flux density) –  $P_N$  (net photosynthetic rate) response curves of *R. delavayi* seedlings under different pH solutions of irrigation.

**Table 3. Comparison of PPF (photon flux density) –  $P_N$  (net photosynthetic rate) response parameters of *R. delavayi* seedlings under different pH solutions of irrigation.**

Treatments	$P_{Nmax}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$L_{CP}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$L_{SP}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$R_d$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$\alpha$
CK	4.74 ± 1.32 <sup>a</sup>	28.67 ± 3.45 <sup>d</sup>	1065 ± 63 <sup>b</sup>	1.03 ± 0.42 <sup>b</sup>	0.0406 ± 0.006 <sup>a</sup>
T <sub>4</sub>	2.64 ± 0.83 <sup>d</sup>	67.59 ± 6.43 <sup>a</sup>	1058 ± 87 <sup>b</sup>	1.05 ± 0.34 <sup>b</sup>	0.0198 ± 0.008 <sup>c</sup>
T <sub>4.5</sub>	3.45 ± 1.05 <sup>c</sup>	59.06 ± 8.24 <sup>a</sup>	1023 ± 69 <sup>b</sup>	1.04 ± 0.22 <sup>b</sup>	0.0211 ± 0.05 <sup>c</sup>
T <sub>5</sub>	4.71 ± 1.16 <sup>a</sup>	32.38 ± 7.52 <sup>c</sup>	982 ± 52 <sup>c</sup>	1.07 ± 0.31 <sup>b</sup>	0.0415 ± 0.06 <sup>a</sup>
T <sub>5.5</sub>	4.79 ± 1.02 <sup>a</sup>	28.80 ± 8.26 <sup>d</sup>	847 ± 62 <sup>d</sup>	1.03 ± 0.28 <sup>b</sup>	0.0401 ± 0.08 <sup>a</sup>
T <sub>6</sub>	4.64 ± 0.98 <sup>a</sup>	35.94 ± 10.15 <sup>c</sup>	1104 ± 84 <sup>a</sup>	1.04 ± 0.23 <sup>b</sup>	0.0326 ± 0.06 <sup>b</sup>
T <sub>6.5</sub>	4.08 ± 1.24 <sup>b</sup>	42.44 ± 9.25 <sup>b</sup>	956 ± 73 <sup>c</sup>	1.11 ± 0.45 <sup>a</sup>	0.0306 ± 0.07 <sup>b</sup>
T <sub>7</sub>	3.69 ± 0.92 <sup>c</sup>	41.58 ± 10.34 <sup>b</sup>	989 ± 67 <sup>c</sup>	1.12 ± 0.37 <sup>a</sup>	0.0321 ± 0.06 <sup>b</sup>

**Table 4. Comparison of net photosynthetic rate ( $P_N$ ), stomatal conductance ( $g_s$ ), transpiration rate ( $E$ ), water use efficiency ( $WUE$ ), and stomatal limitation ( $L_s$ ) of *R. delavayi* seedlings under different pH solutions of irrigation.**

Treatments	$P_N$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$g_s$ ( $\mu\text{mol mol}^{-1}$ )	$L_s$	$E$ ( $\text{mmol m}^{-2} \text{s}^{-1}$ )	$WUE$ ( $\text{mmol mol}^{-1}$ )
CK	4.03 ± 0.53 <sup>b</sup>	0.16 ± 0.05 <sup>b</sup>	0.35 ± 0.04 <sup>c</sup>	3.35 ± 0.75 <sup>a</sup>	1.20 ± 0.74 <sup>c</sup>
T <sub>4</sub>	3.23 ± 0.62 <sup>d</sup>	0.15 ± 0.03 <sup>b</sup>	0.59 ± 0.05 <sup>a</sup>	2.72 ± 0.92 <sup>c</sup>	1.19 ± 0.56 <sup>c</sup>
T <sub>4.5</sub>	3.15 ± 0.45 <sup>d</sup>	0.13 ± 0.04 <sup>b</sup>	0.51 ± 0.04 <sup>a</sup>	2.83 ± 0.68 <sup>c</sup>	1.11 ± 0.62 <sup>c</sup>
T <sub>5</sub>	3.75 ± 0.28 <sup>c</sup>	0.15 ± 0.02 <sup>b</sup>	0.34 ± 0.03 <sup>c</sup>	3.22 ± 0.81 <sup>b</sup>	1.16 ± 0.47 <sup>c</sup>
T <sub>5.5</sub>	4.69 ± 0.57 <sup>a</sup>	0.18 ± 0.03 <sup>a</sup>	0.32 ± 0.01 <sup>c</sup>	3.46 ± 0.77 <sup>a</sup>	1.36 ± 0.72 <sup>b</sup>
T <sub>6</sub>	4.24 ± 0.62 <sup>b</sup>	0.14 ± 0.05 <sup>b</sup>	0.36 ± 0.02 <sup>c</sup>	3.15 ± 0.92 <sup>b</sup>	1.35 ± 0.66 <sup>b</sup>
T <sub>6.5</sub>	4.08 ± 0.43 <sup>b</sup>	0.13 ± 0.04 <sup>b</sup>	0.46 ± 0.03 <sup>b</sup>	2.63 ± 0.86 <sup>c</sup>	1.55 ± 0.59 <sup>a</sup>
T <sub>7</sub>	3.69 ± 0.84 <sup>c</sup>	0.10 ± 0.04 <sup>d</sup>	0.51 ± 0.02 <sup>a</sup>	2.52 ± 0.73 <sup>c</sup>	1.46 ± 0.82 <sup>a</sup>

**Photosynthesis:** Photosynthesis parameters of *R. delavayi* seedlings under different pH solution treatments are shown in (Table 4). The average values of  $P_N$ ,  $g_s$ ,  $L_s$ ,  $E$ , and  $WUE$  of plants were significantly different. Evidently,  $P_N$ ,  $g_s$ , and  $E$  increased initially and then decreased along increasing pH of irrigated solutions, and their maximums were 4.69  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , 0.18  $\mu\text{mol mol}^{-1}$ , and 3.46  $\text{mmol m}^{-2} \text{s}^{-1}$  in T<sub>5.5</sub>, respectively. By comparison,  $L_s$  showed an opposite trend, and the minimum values appeared in T<sub>5.5</sub>. In general,  $WUE$  continuously increased as the pH of irrigated solutions increased.

**Antioxidant enzyme activities and Pro content:** Three antioxidant enzymes, namely, SOD, POD, and CAT, presented a similar pattern of change along the pH gradients of irrigated solutions. As displayed in (Fig. 3), SOD, POD, and CAT continued to increase from T<sub>4</sub>, T<sub>4.5</sub>, T<sub>5</sub>, to T<sub>5.5</sub>, and then decreased to T<sub>7</sub>. In comparison, the change trend of Pro content was opposite along the pH increase of irrigated solutions, and its minimums without significant difference appeared in T<sub>5</sub> and T<sub>5.5</sub>.

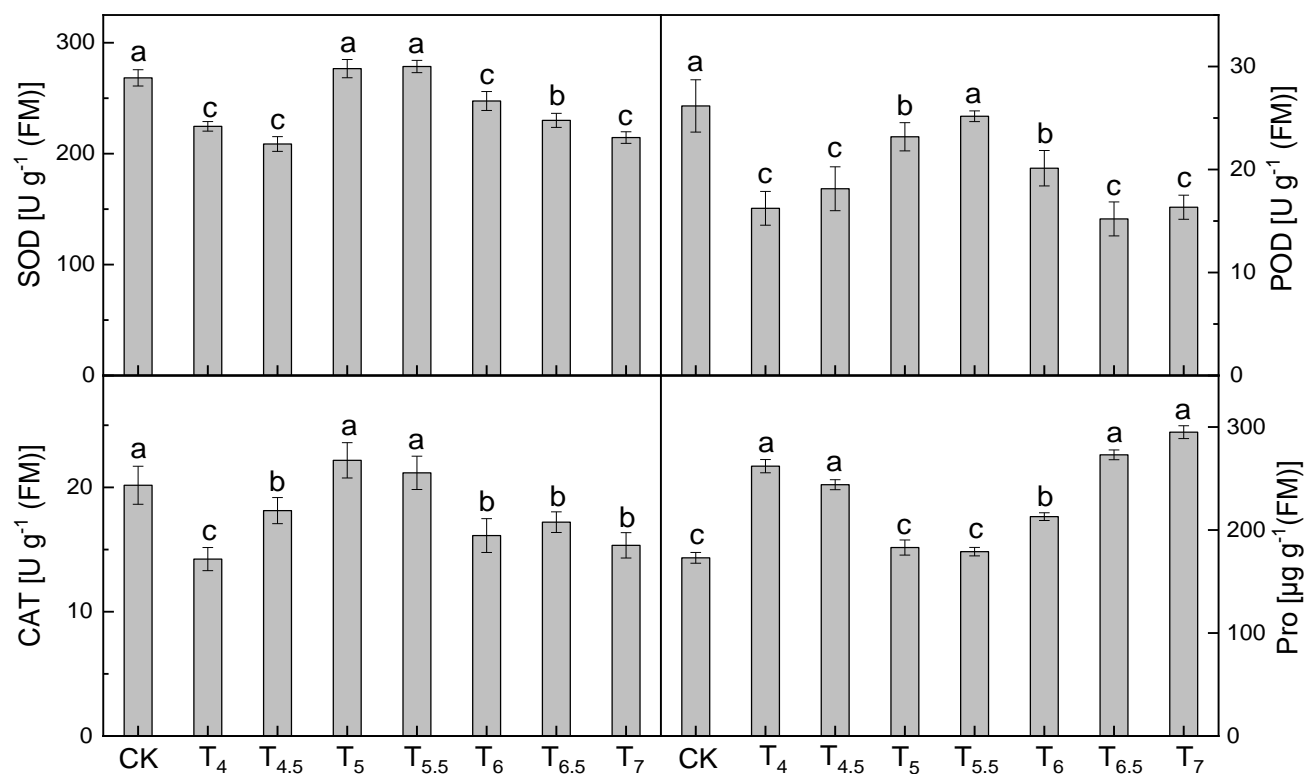


Fig. 3. Changes in superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities, and proline (Pro) content of *R. delavayi* seedlings under different pH solutions of irrigation.

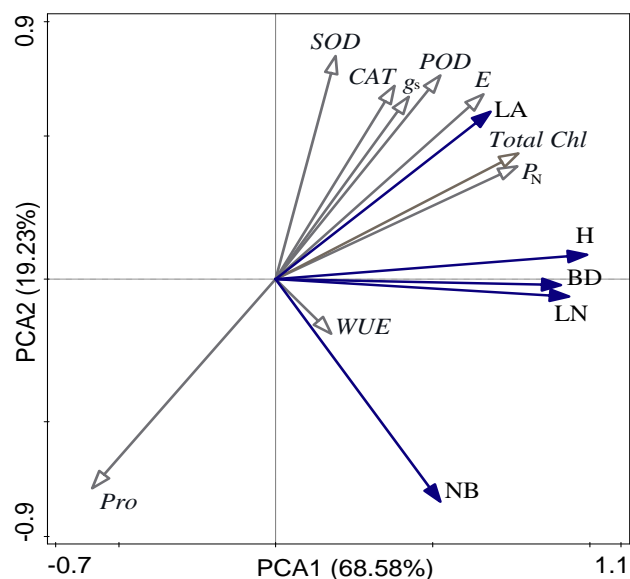


Fig. 4. RDA analysis of growth and development of *Rhododendron delavayi* seedlings and their physio-biochemical parameters under different pH solutions of irrigation.

**RDA analysis between growth and development of seedlings and the physio-biochemical parameters:** The first and second axes of the RDA explained 68.58% and 19.23% of the variance in growth and development of *R. delavayi* seedlings given the effects of the physio-biochemical parameters under different pH solutions of irrigation, respectively (Fig. 4). The growth and development of *R. delavayi* seedlings were affected by  $P_N$ , total Chl,  $E$ , SOD, POD,  $g_s$ , CAT, and  $WUE$ . For

example, H, LN, and BD were more closely associated with  $P_N$  and total Chl. Meanwhile, LA was increased with the increase in the values of SOD, POD, CAT,  $g_s$ , and  $E$ , whereas Pro was reduced. In addition, NB was closely associated with  $WUE$ .

**Discussion**

*R. delavayi* seedlings evidently changed their growth and physiological parameters under different pH solutions of irrigation. Previous research reported that plants of *Rhododendron* family preferred acid soil (Bird, 1921; Fu *et al.*, 2019). In the present research, *R. delavayi* seedlings have presented better growth in T<sub>5.5</sub> and T<sub>6</sub>, as well as several carbon assimilation parameters and three Chl contents. However, significant inhibition of plant growth, such as BD and H, was observed when they were irrigated with strongly acidic (T<sub>4</sub> and T<sub>4.5</sub>), mildly acidic (T<sub>6.5</sub>), or neutral (T<sub>7</sub>) solutions. Simultaneously, their chlorophyll contents were lower than those of CK under the same conditions. These phenomena further indicated that *R. delavayi* had a specific preference for soil acidity and presented different growth and physiological manifestations with pH changes in soil.

Growth and development of *R. delavayi* seedlings had evident preference for soil acidity. The availability of soil nutrient elements had significant difference with change in acidity (Stark *et al.*, 2014). N of soil represents the essential elements for plant growth, and the availabilities of these elements were higher at approximately 6 pH of soil owing to the high activity of nitrogen-fixing bacteria (Bhattacharjee *et al.*, 2008; Soares *et al.*, 2014). *R. delavayi* seedlings had outstanding growth performance, such as BD, H, LN, and LA per

plant in T<sub>5.5</sub> and T<sub>6</sub>. We speculated that *R. delavayi* seedlings might have obtained more N supplements from soil, which were helpful to plant nutrition growth. Similar results were reported by Lee *et al.*, (2005) and White & Scott (2006). Meanwhile, the soil pH values were 5.36 and 5.89 in T<sub>5.5</sub> and T<sub>6</sub>, respectively (Table 1). These values were very close to the suitable acidic range for nitrogen-fixing bacteria survival (Lipa & Janczarek, 2020). Therefore, soil had better ability to supply nutrient to improve the growth of *R. delavayi* seedlings in T<sub>5.5</sub> and T<sub>6</sub> treatments. During the course of the experiment, the BN number per plant had no significant difference, which could be due to the slow branch development and the days of experiment observation.

Significant difference in Chl contents was found among the plants irrigated with different pH solutions. Chl content is a reference index to characterize the light energy utilization ability of plants (Wei *et al.*, 2019). Chl *a* mainly absorbs red light, and Chl *b* mainly absorbs blue and purple light (Hu *et al.*, 1998). In addition, Ch (a/b) is a critical indicator of shade tolerance for green plants (Luimstra *et al.*, 2019). The contents of Chl *a*, Chl *b*, and total Chl in T<sub>5.5</sub> were significantly higher than CK. Moreover, they were significantly lower in T<sub>4</sub>, T<sub>4.5</sub>, and T<sub>7</sub> than in CK. This finding indicated that the solution of pH 5.5 could significantly increase the chlorophyll synthesis of *R. delavayi*. However, extremely acidic or nearly neutral soil (pH < 4.56 or > 6.85) was un conducive to the accumulation of photosynthetic pigment. Similar results were observed by Shah *et al.*, (2001). Moreover, Ch (a/b) tends to increase with increasing soil pH, thereby indicating that the shade tolerance of *R. delavayi* tended to decrease. This result is greatly important for regulating the PPFD of *R. delavayi* growth.

Photosynthetic ability and biochemical parameters of *R. delavayi* were significantly affected by the pH change in the irrigated solutions. The PPFD-*P<sub>N</sub>* response curves of *R. delavayi* seedlings well reflected the differences of carbon assimilation potential under different pH solutions of irrigation. According to the fitting values of PPFD-*P<sub>N</sub>* response curves, *R. delavayi* had the highest carbon assimilation potential in T<sub>5</sub>, T<sub>5.5</sub>, and T<sub>6</sub>, whereas *L<sub>CP</sub>*, *L<sub>SP</sub>*, and *R<sub>d</sub>* were the lowest in T<sub>5.5</sub>. These findings showed that the low light utilization ability of *R. delavayi* was significantly increased with the low respiratory expenditure under T<sub>5.5</sub> treatment. Simultaneously, the higher *α* indicated rubisco more efficiently catalyzed photosynthesis. Meanwhile, photosynthesis parameters demonstrated that *R. delavayi* had the highest physiological activity in T<sub>5.5</sub>. In general, plants grew more vigorously in T<sub>5.5</sub>.

Antioxidant enzyme activities and Pro content reflected the stress tolerance and survival status of plants (Qiu *et al.*, 2020). Meanwhile, changes in antioxidant enzyme activities and Pro content often indicated the degree of plants to resist osmotic stress (Lü *et al.*, 2019). In our study, the higher activities of SOD, POD, and CAT appeared under T<sub>5</sub> and T<sub>5.5</sub>, which indicated that *R. delavayi* seedlings grow well and have certainly antioxidant capacity. Synchronously, the lowest Pro content showed plants did not suffer from obvious stress conditions. On the contrary, *R. delavayi* seedlings irrigated with lower (T<sub>4</sub> and T<sub>4.5</sub>)

and higher (T<sub>6.5</sub> and T<sub>7</sub>) pH solutions accumulated more proline and lower antioxidant enzyme, compared with CK. It was clear that *R. delavayi* seedlings were unable to effectively activate the reactive oxygen scavenging system through self-physiological regulation, although plants experience adversity. The similar results were observed in *R. catawbiense* and *R. calophytum* (Wang *et al.*, 2009; Ran *et al.*, 2010).

The correlations certainly existed among growth, development, and physio biochemical indexes for *R. delavayi*. According to results of DCA analysis, the higher total Chl contents and *P<sub>N</sub>* indicated a promoted utilization of light energy and the rate of carbon assimilation (Li *et al.*, 2013; Qiu *et al.*, 2020), thereby rapidly increasing H, BD, and LN of *R. delavayi*. Meanwhile, LA had a positive relationship to the protective enzyme activity and *E*, confirming that expanding the leaf was beneficial to improve plant resistance and water transpiration. Similarly, a large number of BN could promote leaf development to synthesize more carbohydrates. This approach is effective for plants to improve *WUE*.

## Conclusion

When *R. delavayi* seedlings were continuously irrigated with different pH solutions for two months, we determined the growth, photosynthetic, and biochemical parameters of plants, including the soil pH, to filter out the suitable soil acidity for *R. delavayi* survival. The results showed that *R. delavayi*, which was irrigated with the pH 5.5 solution, had relatively superior growth and development advantages. Synchronously, the pH of the matrix was 5.36. Furthermore, the rich Chl content, high *P<sub>N</sub>*, large LA, and developed BN of *R. delavayi* seedling contributed to the rapid growth and resistance improvement of individuals. Therefore, the information will promote our understanding of the adaptability of *R. delavayi* to soil acidity and assist in efficient artificial cultivation of *R. delavayi* seedling.

## Acknowledgements

This research was supported by the National Science Foundations of China (No. 31960320) and Top discipline construction of ecology [GNYL (2017)009].

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(Received for publication 6 July 2021)