



Original Research Article

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Effects of different treatments of pH on growth and photosynthesis of *Phoebe bournei* seedlings

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Abstract

To provide theoretical basis for the cultivation and landscape application of *Phoebe bournei* seedlings, the photosynthetic and physiological characteristics of the seedlings were determined in the experience. In this study, the growth, photosynthesis and physiological indexes of *P. bournei* seedlings under different pH conditions were tested every 15 days for a total of 45 days by using 2-year-old seedlings with different solutions with pH of 4.0, 5.0, 6.0, 7.0, 8.0, 8.5 and 9.0 as 7 different irrigation treatments. The results showed that the growth of *P. bournei* seedlings grew significantly in acid environment and inhibited in alkaline environment, and the growth rate of plant height in acid environment is significantly higher than natural condition of pH7.0. The chlorophyll and carotenoid contents increase with the increase of acid, and decrease in alkaline environment above pH8.0, which indicate that *P. bournei* seedlings has better adaptability to acid environment than alkaline environment. The net photosynthetic rate (Pn) and transpiration rate (Tr) of *P. bournei* seedlings reach the highest level at pH6.0, and the stomatal conductance (Gs) and intercellular CO₂ concentration (Ci) reach the highest level at pH 4.0. However, these indicators are low in alkaline environment above pH 8.0. Under acid stress, all physiological indexes of *P. bournei* are increased; under alkaline stress, the superoxide dismutase (SOD) activity and the soluble protein (SP) content show a trend of increasing, and the peroxidase (POD) activity, MDA content and proline (Pro) show a trend of decreasing later under the treatment of pH 8.5 ~ 9.0. According to all the above, *P. bournei* seedlings has good tolerance to acid environment, and the suitable pH value of solution for *P. bournei* seedlings growth is pH 5.0-8.0.

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Introduction

Phoebe bournei, the genus *Phoebe* of *Lauraceae*, is evergreen in all season. It is a superior timber tree species, with durable wood material and a special aroma, after processing showing a unique metal luster.

P. bournei is one of the precious tree species in China, and is endemic to China and threatened by habitat loss (Chen et al., 2018). Thus *P. bournei* is protected as the secondary protected plants as well (Fang et al., 2000). *P. bournei* is an excellent tree species for urban landscaping because of its beautiful shape, straight

trunk and long and narrow leaves. It is a good tree species for afforestation because of its strong root germination ability, long life span, few diseases and insect pests and strong tolerance. The researches about *P. bournei* in China includes cultivation and management of *P. bournei* (Li et al., 2015; Wang et al., 2019; Dong et al., 2014), the effects of shading on photosynthesis (Tang et al., 2019), cutting and tissue culture techniques of *P. bournei* (Xiao et al., 2020; Qu et al., 2010) and the effects of water stress on physiological and biochemical responses of *P. bournei* seedlings (Ge et al., 2014). However, there are few researches on the adaptability of *P. bournei* to soil pH, only about the effects of acid environment on growth of *Phoebe* seedlings (Liu, 2020). In order to provide theoretical basis for protecting *P. bournei* better, the photosynthetic physiological characteristics of *P. bournei* seedlings were studied under different pH conditions, and the soil acid-base range suitable for its growth and development was found in this experiment.

Materials and methods

General situation of the test site

The bonsai garden in the West Campus of Yangtze University in Jingzhou City, Hubei Province was selected as the outdoor experimental site, where locates in the south central part of Hubei Province. The climate in Jingzhou is North subtropical monsoon humid climate. Jingzhou has abundant sunshine and heat, long frost free period, sufficient water resources, dense rivers and lakes, and abundant rainfall, with annual rainfall of 1100 ~ 1300mm. It is suitable for the cultivation and growth of crops.

Test material and design

In March 2016, 2-year-old seedlings of *P. bournei* with similar plant height and diameter were bare-root transplanted and placed in the Bonsai Garden for 3 months. During the 3 months, normal fertilizer and water management of seedlings was done. In June 2016, *P. bournei* with similar growth (23 ± 0.5 cm in height and 0.5 ± 0.2 cm in diameter) was selected and irrigated with 200 ml 1 time Hoagland nutrient solution from 7:00 am to 9:00 am daily. The pH value was adjusted by 1 mol/L NaOH and HCl solution. The pH values from low to high were 4.0, 5.0, 6.0, 7.0, 8.0, 8.5 and 9.0 and labelled M1 to M7. Every 15 days, every indicator repeated 3 times.

Indicator determination and method

(1) **Morphological index:** plant height, ground diameter and leaf area index of seedlings were measured every 15 days (The third and fourth well-grown leaves from top to bottom were selected and calculated by grid method).

(2) **Photosynthetic pigment content:** according to the method of Zhu et al. (2013), the photosynthetic pigment content was determined by ultraviolet spectrophotometer.

(3) **Photosynthetic index:** the net photosynthetic rate, transpiration rate, stomatal conductance and intercellular CO₂ concentration of *P. bournei* were measured by Li-Cor6400 portable photosynthetic apparatus according to the method of Chen et al. (2019).

(4) **Physiological indexes:** the activity of superoxide dismutase (SOD) and peroxidase (POD) were determined by nitroblue tetrazolium method and guaiacol colorimetry (Li, 2002); malondialdehyde (MDA), soluble protein (SP) and proline (pro) were measured by thiobarbituric acid method, Coomassie brilliant blue method and acid ninhydrin method (Liu, 2018).

Data analysis

SAS 8.0 software was used to analyze the difference among different treatments of *P. bournei*. Excel 2016 and SigmaPlot 13.0 software were used to organize data and draw charts

Results

Effects of different pH solutions on the growth of *P. bournei* seedlings

The results of Figs.1 ~ 3 show that compared with neutral treatment (M4), *P. bournei* seedlings grow faster under acid treatments (M1~M3), but slower under alkaline treatments (M5~M7). Among them, the difference of plant height was the most obvious (Fig. 1). Under the acid treatment pH5.0 (M2), the plant height of *P. bournei* seedlings increased fastest and was at the highest level in the experiment, which reached the highest value of 37.11 cm on day 45 and increased 62.12% compared with day 0. The plant height of M4 increased from 23.20 cm (day0) to 27.85 cm (day 45), with an increase of 20.04%. In the

day 45 of experiment, the difference between M2 and M4 reached 9.26 cm, the difference was obvious; the difference between M1 and M3 was not obvious, only 1.08 cm, showing significant difference with the control group, which was 6.32 cm and 5.24 cm higher than that of the control group (M4). Under M6 (pH 8.5), the growth of plant height of *P. bournei* was at the slowest level and only 3.06 cm higher than the starting values on day 45 (increase was 13.23%). Besides, the height value was 3.74

cm higher than M4, 10.06 cm, 10.92 cm and 8.98 cm lower than M1, M2 and M3, respectively. In the experiment, different pH solutions had little effect on the ground diameter, and the difference was not significant (Fig. 2). The leaf area index of *P. bournei* increased slowly with the increase of alkaline solution, and accelerated with the increase of acidic solution, but there was no significant difference among these treatments (Fig. 3).

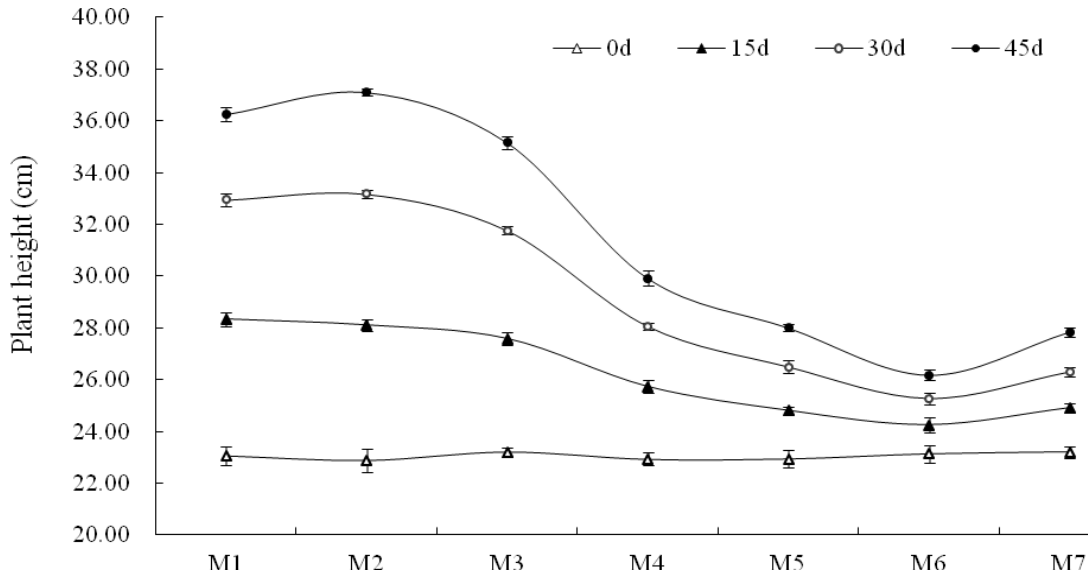


Fig. 1: The plant height of *P. bournei* seedlings in different treatments.

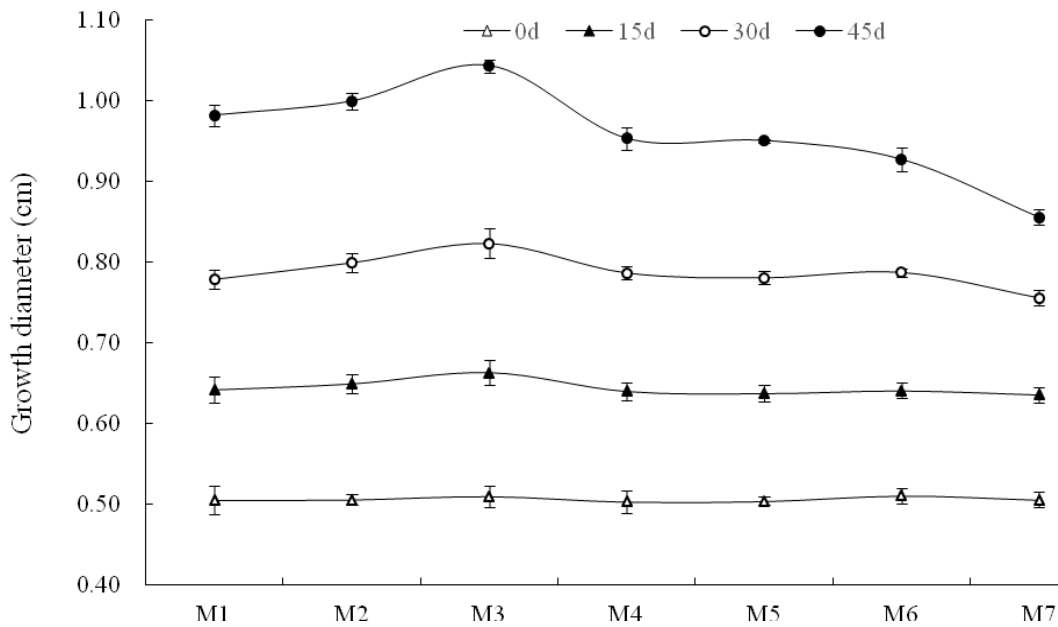


Fig. 2: The growth diameter of *P. bournei* seedlings in different treatments.

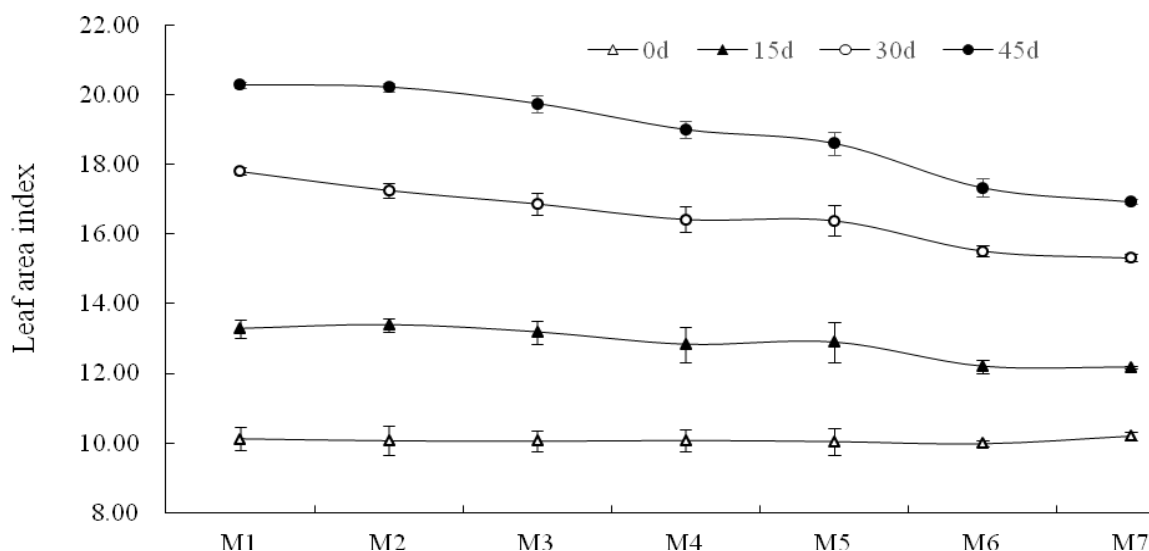


Fig. 3: The leaf area index of *P. bournei* seedlings in different treatments.

Table 1. The pigment contents of *P. bournei* seedlings in different treatments.

Treatment	Chl a/(mg·g ⁻¹)		Chl b/(mg·g ⁻¹)		Chl/(mg·g ⁻¹)		Car/(mg·g ⁻¹)	
	15 d	45 d	15 d	45 d	15 d	45 d	15 d	45 d
M1	1.30±0.13a	1.29±0.07a	0.92±0.15b	0.66±0.04a	0.42±0.06a	0.43±0.02a	2.21±0.35a	1.93±0.11a
M2	0.90±0.18b	1.17±0.04ab	0.50±0.05d	0.60±0.02ab	0.31±0.03b	0.41±0.01ab	1.38±0.26b	1.76±0.06ab
M3	0.56±0.07c	0.95±0.14cd	0.28±0.05e	0.50±0.06bc	0.21±0.02c	0.35±0.05bc	0.83±0.16c	1.44±0.15de
M4	1.05±0.06b	0.84±0.08d	0.55±0.04cd	0.44±0.04c	0.37±0.02ab	0.30±0.03c	1.59±0.10b	1.27±0.12e
M5	0.62±0.07c	1.04±0.12bc	1.64±0.22a	0.54±0.10bc	0.21±0.03c	0.38±0.05ab	2.20±0.27a	1.58±0.20bcd
M6	0.99±0.12b	0.99±0.11cd	0.59±0.05cd	0.52±0.05bc	0.36±0.04ab	0.39±0.03ab	1.58±0.27a	1.50±0.16cde
M7	1.11±0.01ab	1.24±0.13a	0.75±0.09bc	0.51±0.09bc	0.42±0.01a	0.38±0.06ab	1.84±0.10ab	1.74±0.32abc

Notes: n=21, each treatment was replicated three times. Error bars are means and standard error for the mean of three replications. Different lowercase letters mean significant difference ($P<0.05$), the same below.

Effects of different pH solutions on the photosynthetic pigment content of *P. bournei* seedlings

The results of Table 1 show that the photosynthetic pigment content of *P. bournei* seedlings first increased and then decreased with the increase of stress time. Under the treatment of strong acid treatment pH 4.0 (M1) and strong alkaline treatment pH 9.0 (M7), the content of photosynthetic pigments was significantly higher than the control group (M4), and the content of each photosynthetic pigment was at the highest level all the time under the treatment M4. In the day 45 of experiment, the content of Chl a, Chl b, Chl, and Car respectively reached 1.29 mg·g⁻¹, 0.66 mg·g⁻¹, 0.43 mg·g⁻¹, 1.93 mg·g⁻¹ and 0.45 mg·g⁻¹, 0.22 mg·g⁻¹, 0.13 mg·g⁻¹, 0.66 mg·g⁻¹ higher than control group (M4) under the acid treatment pH4.0 (M1). These indexes respectively reached 1.24 mg·g⁻¹, 0.51 mg·g⁻¹, 0.38 mg·g⁻¹, 1.74 mg·g⁻¹ and 0.40 mg·g⁻¹, 0.07 mg·g⁻¹, 0.08 mg·g⁻¹, 0.47 mg·g⁻¹ higher than control group (M4)

under the alkaline treatment pH9.0 (M7). Besides, there was no significant difference between the treatment pH6.0 (M3) and the control (M4) for day45, which were 0.11 mg·g⁻¹, 0.06 mg·g⁻¹ and 0.05 mg·g⁻¹ higher than the Chl a, Chl b, Chl, and Car contents of *P. bournei* seedlings of treatment M4. Also, there was no significant difference between the content of Chl b and Chl under alkaline treatments (M5 ~ M7), and there was no significant difference in carotenoid content between pH 5.0 (M2) and pH 9.0 (M7), pH 6.0 (M3) and pH 8.5 (M6).

Effects of different pH solutions on the photosynthetic index of *P. bournei* seedlings

The results of Figs. 4~7 show that the net photosynthetic rate (Pn), transpiration rate (Tr) and stomatal conductance (Gs) of *P. bournei* seedlings first increased, then decreased and finally increased with the increase of stress time. In the day 45 of experiment, Pn of *P. bournei* seedlings reached the highest level 10.1225 μmol·m⁻²·s⁻¹ under the treatment pH 6.0 (M3)

and $3.49 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ significantly higher than control group (M4). There was no significant difference between the alkaline treatment pH 9.0 (M7) and the control group (M4) for Pn value of *P. bournei* seedlings, only $0.2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Under the treatments of pH 4.0 (M1), pH 8.0 (M5), pH 8.5 (M6), there was no significant difference with M4 (Fig. 4). The trend of Tr value and Gs value was similar (except for the treatment pH 4.0), and the most significant difference was found

between the control group (M4) and the alkaline treatment pH 9.0 (M7) on day 45, which were 65.89% and 49.27% of M4 (Figs. 5~6). The results showed that the intercellular CO_2 concentration (C_i) of *P. bournei* showed obvious regularity on day 45: $M1 > M2 > M3 > M4 > M5 > M6 > M7$, and reached the lowest value of $216.08 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ under the treatment pH 9.0 (M7), which was $48.505 \text{ mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ significantly lower than M4 (Fig. 7).

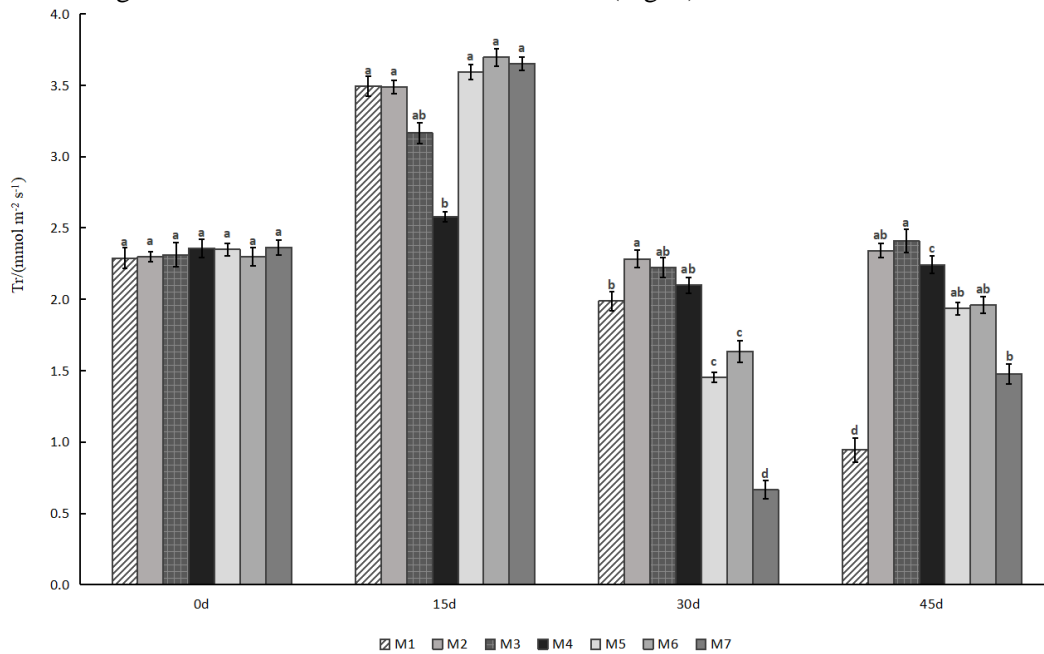


Fig. 5: The transpiration rate of *P. bournei* seedlings in different treatments.

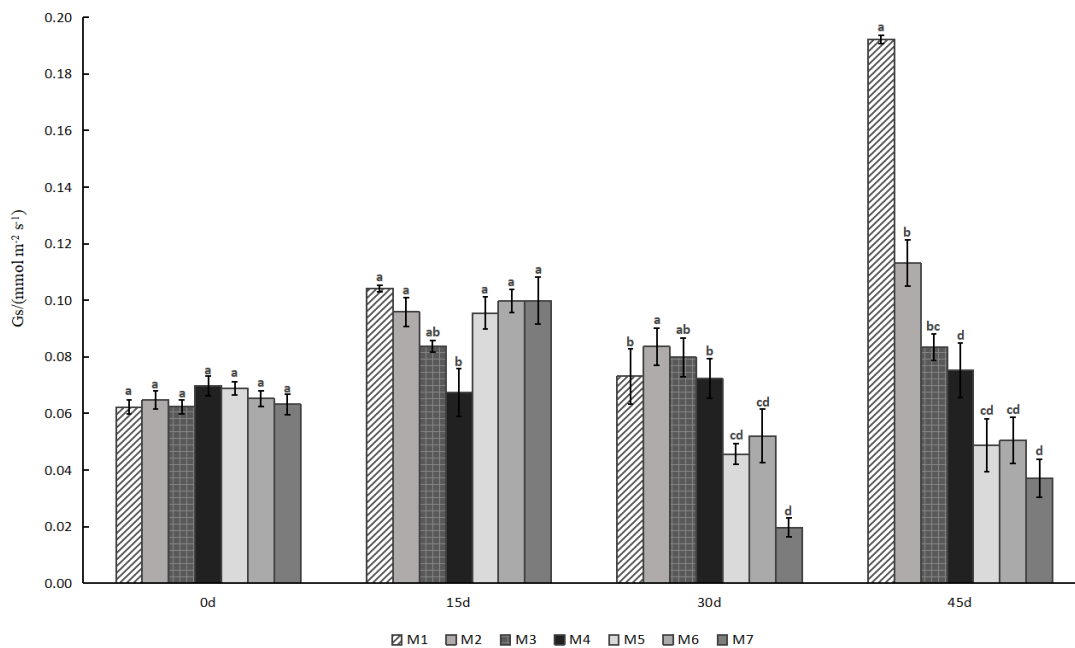


Fig. 6: The stomatal conductance of *P. bournei* seedlings in different treatments.

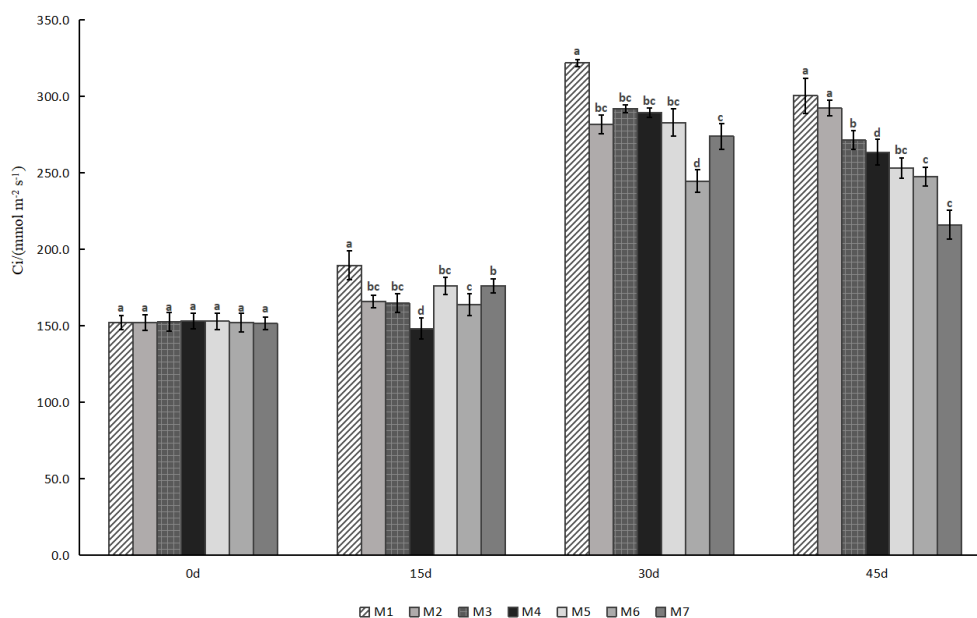


Fig. 7: The intercellular CO₂ concentration of *P. bournei* seedlings in different treatments.

Effects of different pH solutions on physiology of *P. bournei* seedlings

Effects of different pH solutions on the SOD activity of *P. bournei* seedlings

The results of Fig. 8 show that with the increase of stress time, the SOD activity of *P. bournei* seedlings in all treatments showed an upward trend and reached the highest level on day 45, while the control group

(M4) was at the lowest level all the time during the experiment. From day 15, the SOD activity increased with the increase of alkalinity under the alkaline treatment pH 8.0~9.0 (M5~M7), and reached the highest level 7.98 U·g⁻¹·min⁻¹ under the treatment pH 9.0 (M7) on day 45, which was 1.40 times higher than the control group (M4). The SOD activity under acid treatment (M1~M3) was lower than that under alkaline treatment (M5~M7).

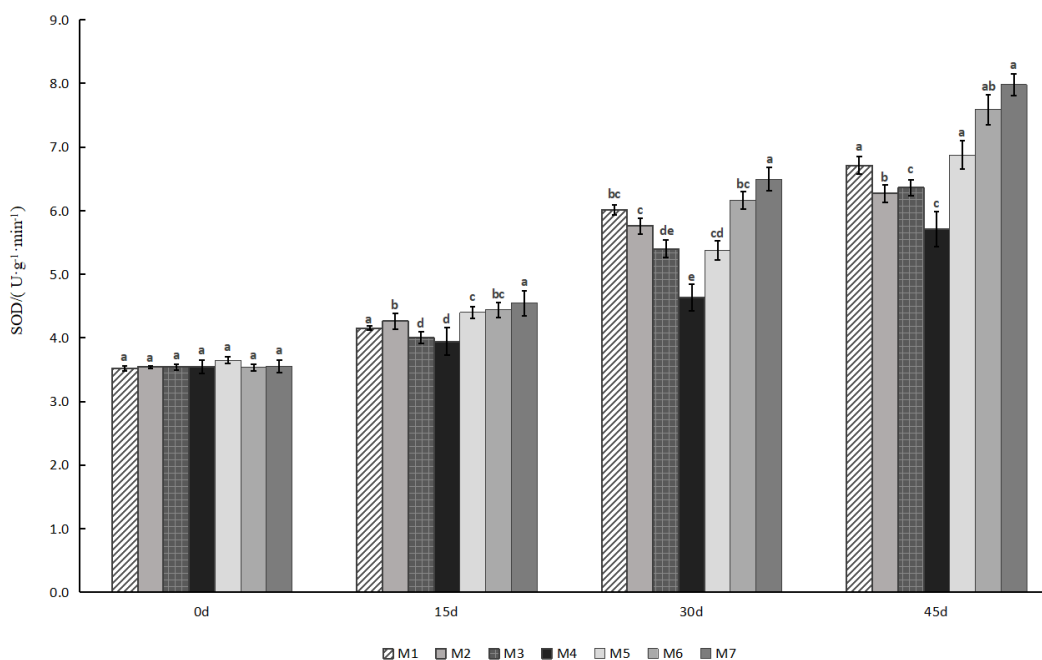


Fig. 8: The SOD activity of *P. bournei* seedlings in different treatments.

Effects of different pH solutions on the POD activity of *P. bournei* seedlings

The results of Fig. 9 showed that the POD activity of *P. bournei* increased under acid and alkaline treatments. Under the treatment pH 9.0 (M7), POD activity reached the highest level of $9.64 \text{ U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ on day 30, which was 1.16 times of the control group (M4). From the day 15 in the experiment, the POD activity under neutral treatment M4 was at the lowest level, and increased with the increase acid and alkaline treatments; by the end of the experiment, POD activity of *P. bournei* increased with the increase of acidity under the acid treatments (M1~M3), and reached the highest value of $9.09 \text{ U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ under the treatment pH 4.0 (M1), which was $0.58 \text{ U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$ higher than M4. Besides, there was no significant difference between the alkaline treatment (M5~M7) and M1, and the difference value was less than $0.1 \text{ U}\cdot\text{g}^{-1}\cdot\text{min}^{-1}$.

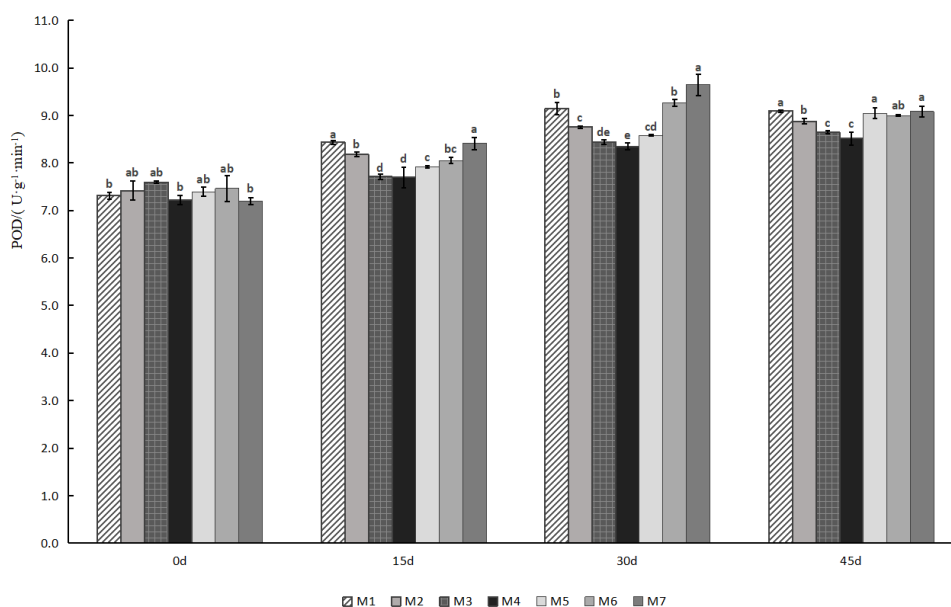


Fig. 9: The POD activity of *P. bournei* seedlings in different treatments.

Effects of different pH solutions on the SP content of *P. bournei* seedlings

The results of Fig. 11 show that SP content of *P. bournei* seedlings increased in different degrees with the increase of stress time. Early in the experiment, the increase of SP content under all treatments was small and there was no significant difference among them. At the end of the experiment, the SP content showed obvious regularity: SP content reached the lowest value under neutral treatment and increased

Effects of different pH solutions on the MDA content of *P. bournei* seedlings

The results of Fig. 10 showed that acid-base stress has a significant effect on MDA content of *P. bournei* seedlings. With the increase of stress time, the MDA content increased under the treatments pH 4.0 (M1), pH 5.0 (M2), pH 8.0 (M5), and reached the highest level on day 45, which were 149.66%, 169.24% and 149.66% of the control group (M4). The MDA content under the treatments pH 8.5 (M6) and pH 9.0 (M7) decreased in the later experiment, while still $4.82 \mu\text{mol}\cdot\text{g}^{-1}$ and $3.79 \mu\text{mol}\cdot\text{g}^{-1}$ higher than M4. There was no significant difference between the acid treatment pH 6.0 (M3) and the control group (M4), but it was significantly lower than other treatments. The results showed that the membrane of *P. bournei* seedlings suffered from oxidative damage under acid-base stress, and the degree of damage was small under the pH treatments 5.0 ~ 6.0.

with the increase of acidity and alkalinity. Also, SP content under acid treatment was slightly higher than that under alkaline treatment. Under the treatment pH 4.0 (M1), SP content reached the highest value of $392.13 \text{ mg}\cdot\text{g}^{-1}$. There was no significant difference between M1 and M7, and M1 and M7 respectively $43.23 \text{ mg}\cdot\text{g}^{-1}$ and $40.97 \text{ mg}\cdot\text{g}^{-1}$ higher than M4 ($348.90 \text{ mg}\cdot\text{g}^{-1}$). Besides, there was no significant difference of SP content between control group (M4) and the treatments of pH 6.0 (M3), pH 8.0 (M5) and pH 8.5 (M6).

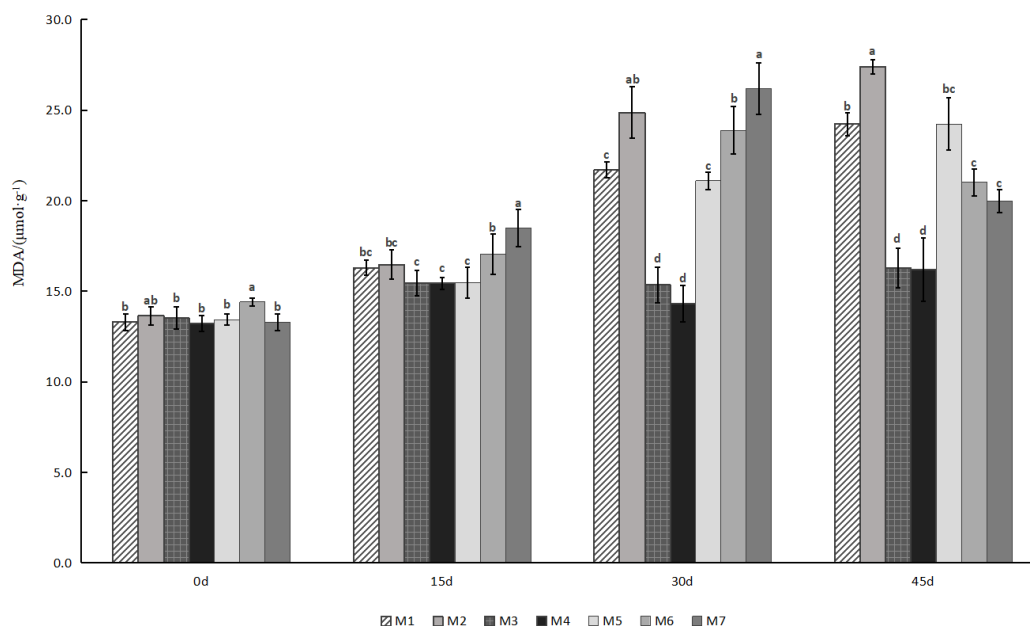


Fig. 10: The MDA content of *P. bournei* seedlings in different treatments.

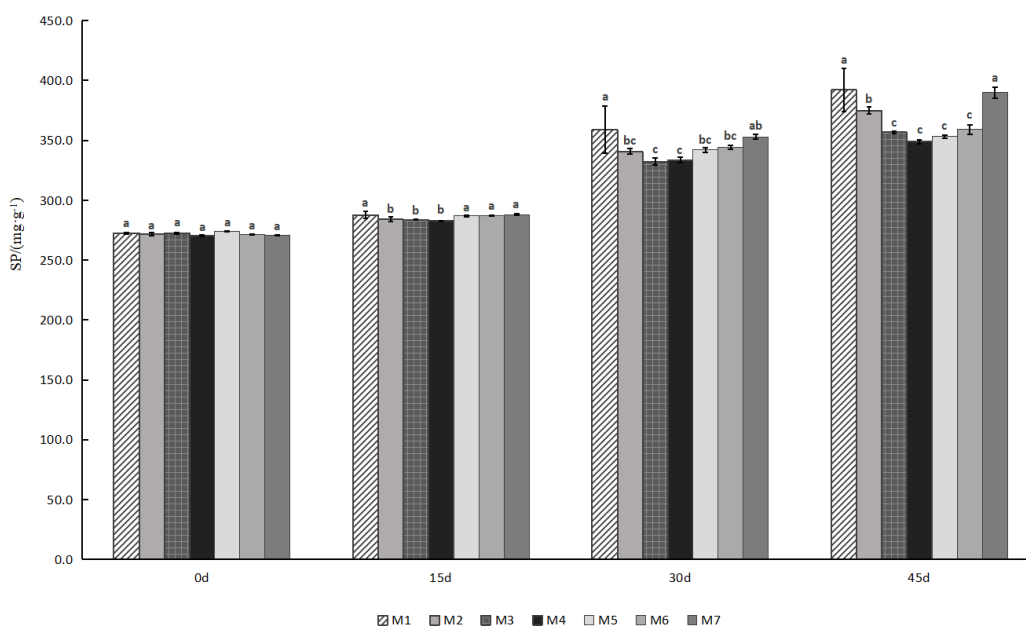


Fig. 11: The soluble protein content of *P. bournei* seedlings in different treatments.

Effects of different pH solutions on the Pro content of *P. bournei* seedlings

The results of Fig. 12 show that with the increase of stress time, the Pro content of *P. bournei* seedlings under acid treatments (M1~M3) continuously increased, and the Pro content from high to low was M1 > M2 > M3 all the time in the experiment. Under the alkaline treatments (M5~M7), the Pro content showed a growth

trend in the early experiment, and M6, M7 decreased in the later experiment, while Pro content still higher than the control group (M4). In the day 45 of the experiment, the pro content of *P. bournei* seedlings was in direct proportion to acidity and in inverse proportion to alkalinity, and there was difference to a certain extent among the treatments. Under the acid treatment pH 4.0 (M1), the Pro content reached the highest value of 26.861 µg·g⁻¹.

Besides, there was significant difference that M1 and M2 were respectively $8.838 \mu\text{g}\cdot\text{g}^{-1}$, $5.2 \mu\text{g}\cdot\text{g}^{-1}$ higher than the control group M4 ($18.023 \mu\text{g}\cdot\text{g}^{-1}$). The treatment M3 was $0.31 \mu\text{g}\cdot\text{g}^{-1}$ lower than M4 with little difference. There was no significant difference among

the alkaline treatments (M5~M7) of the Pro content, and M1, M2 had little difference with that under the treatment pH8.0 (M5), but had significant difference with the control group (M4), which was $5.211 \mu\text{g}\cdot\text{g}^{-1}$ higher than M4.

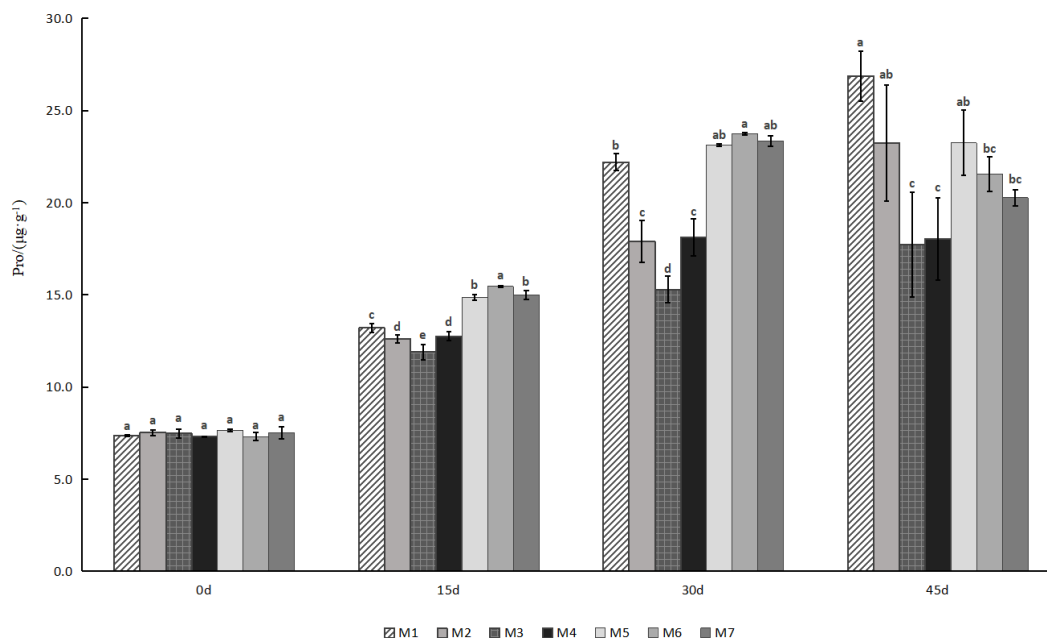


Fig. 12: The proline content of *P. bournei* seedlings in different treatments.

Discussion

The past researches indicated that plants grow and developed well under weak acid and weak alkaline environment, while growth and development were inhibited under strong acid and strong alkaline environment (Tian et al., 2020; Li et al., 2020; Zhou et al., 2018; Mei et al., 2019). The results showed that the growth and development of *P. bournei* seedlings under acid environment was significantly better than that under alkaline environment. Besides, the plant height ran up to the top level under pH 5.0 treatment. The ground diameter reached the highest value under pH 6.0 treatment, and the leaf area index increased most significantly under pH 4.0 treatment. However, the growth and development of *P. bournei* seedlings was weak under strong alkaline environment. The reason is that under acid environment, light capture area of leaves increases, which increases the transmission rate of photosynthetic electron and the accumulation of organic matter. Thus, the growth and development of *P. bournei* was promoted (Zhao et al., 2013). The results indicated that *P. bournei* has certain tolerance to acid environment.

The photosynthetic pigment content of plants is directly related to their absorption of light energy (Hu, 2019). The strong acid and strong alkaline environment will damage the chloroplast, resulting in the reduction of photosynthetic pigment and the decrease of photosynthesis, while the weak acid and weak alkaline environment can promote the photosynthesis of plants to a certain extent (Li, 2002). In this experiment, the contents of Chl a, Chl b, Chl and Car content of *P. bournei* seedlings reached the highest level under pH 4.0 treatment, followed was pH 5.0 and pH 9.0 treatments, and reached the lowest level under pH7.0 treatment. It is consistent with the results of Yi Xiaoqin et al. Studied on the effects of simulated acid rain stress on the growth and photosynthetic physiology of tea seedlings (Yi, 2019). *P. bournei* leaf was damaged under strong acid and alkaline environment, while adjusted more to pH 5.0 ~ 8.0 environment.

The results of photosynthetic index showed that *Phoebe bournei* had a certain acid resistance and photosynthesis was strong under acid environment, which was consistent with the research results of Yu et al. (2011) that acid treatment can promote the utilization rate of *P.*

bournei to light energy. In this experiment, the Pn and Tr values of *Phoebe bournei* seedlings were the highest under weak acid environment pH6.0 and the lowest under strong acid environment pH4.0. By the end of the experiment, the Pn values of *P. bournei* seedlings under pH6.0 and pH9.0 treatments were significantly higher than that under pH7.0 treatment, and the Tr values under pH4.0 and pH9.0 treatments were significantly lower than that under the control. The Gs and Ci values were the highest under pH 4.0 treatment and lowest under pH 9.0 treatment.

Under strong acid and alkaline environment, the membrane system of plant cells is destroyed. In order to remove the damage caused by peroxides, plants will form POD and SOD to remove excessive active oxygen. As a product of lipid membrane peroxidation, MDA will accumulate a lot and damage plant cells when the balance of active oxygen is destroyed (Ding et al., 2020). In this experiment, SOD activity under alkaline environment was significantly higher than that under acid environment, and POD activity had no significant difference under acid-base stress. In the early experiment, the MDA content of *P. bournei* seedlings increased significantly under the pH 4.0 ~ 5.0 and pH 8.0 ~ 9.0 environment, which indicated that the strong acid and alkaline environment had damaged the plasma membrane permeability of *P. bournei* seedlings. However, in the later experiment, the MDA content decreased significantly under pH 8.5 ~ 9.0 environment, which was inconsistent with the research results of Huang et al. (2020). The reason is that the continuous strong alkaline environment greatly destroyed the cell membrane of *P. bournei* and seriously affected the biosynthesis of *P. bournei*, thus reducing the synthesis of malondialdehyde.

Under acid-base stress, plants adjust the content of osmotic substances to resist the damage caused by harsh environment, while Pro and SP can promote osmotic pressure and maintain the stability of protoplast colloid (Yuan et al., 2019). In the experiment, SP content and Pro content of *P. bournei* seedlings decreased first and then increased with the increase of pH value of solution, which was consistent with the results of Yuan et al. (2019). Among them, SP content of *P. bournei* increased significantly under pH4.0 and pH9.0 treatment, which may be due to the cell membrane function of *P. bournei* was damaged and the growth of *P. bournei* was inhibited. However, SP content of *P. bournei* increased of a different degree under pH 5.0 ~

8.5 treatments, which showed *P. bournei* had certain resistance to pH 5.0 ~ 8.5 environments. The Pro content was the highest level under pH 4.0 environment, but gradually decreased under pH 8.0 ~ 9.0 environment in the later experiment. It indicated that alkaline environment caused serious damage to *P. bournei* seedlings, which was consistent with the research results of Zhu et al. (2015).

Conclusions

In conclusion, the results showed that *P. bournei* seedlings grew faster in the acid environment with pH 4.0 ~ pH6.0 and slower in the alkaline environment with pH 8.0 ~ pH 9.0. Besides, the photosynthetic physiology of *P. bournei* seedlings was inhibited in strong acid environment with pH 4.0 and alkaline environment with pH above 8.0. The results showed that pH 5.0 ~ 8.0 was the suitable pH range for *Phoebe bournei* seedlings.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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