

Effects of Different Treatments on the Survival Rate and Physiological Characteristics of Bare-Root Transplanted *Phoebe zhennan* and *Phoebe chekiangensis* (Postprint)

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Abstract

To investigate the effects of combined water-retaining agent and ABT treatment on the transplant survival rate and physiological characteristics of bare-root seedlings of *Phoebe zhennan* (ZN) and *Phoebe chekiangensis* (ZJN), this study employed three different concentrations of water-retaining agent and ABT to jointly treat ZN and ZJN bare-root seedlings, measured the seedling survival rate and physiological indices after transplanting, and conducted a comprehensive evaluation of each index to assess seedling recovery under different treatment conditions. The results showed that, compared with the control group, the survival rates of ZN and ZJN were significantly improved after combined treatment; the number of new leaves in ZN increased significantly, whereas that in ZJN showed no increase; the contents of chlorophyll and carotenoids were significantly enhanced, with more pronounced effects at the ZN1 or ZJN1 level; the ratios of $(GA_3+ZR+IAA)/ABA$ and IAA/ABA were significantly increased, but no significant differences were observed among different concentration treatments for ZN, while ZJN1 was significantly higher than the ZJN2 treatment group; the contents of soluble protein and soluble sugar both decreased. Comprehensive evaluation analysis using the membership function indicated that the performance of transplant survival rates of bare-root seedlings followed the order of $ZN1 > ZN2 > CK$ and $ZJN1 > ZJN2 > CK$, respectively. In conclusion, the combined treatment with water-retaining agent and ABT promoted the transplant survival of ZN and ZJN bare-root seedlings, with the ZN1 or ZJN1 level being superior.

Full Text

Effects of Different Treatments on Survival Rate and Physiological Characteristics of *Phoebe zhennan* and *Phoebe chekiangensis* Seedlings with Bare-Root Transplanting

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Abstract

This study investigated the effects of combined treatments with three different concentrations of super-absorbent polymer and ABT rooting powder on the survival rate and physiological characteristics of bare-root seedlings of *Phoebe zhennan* (ZN) and *P. chekiangensis* (ZJN). Bare-root seedlings were treated with the combined formulations, and post-transplant survival rates and physiological indices were measured. A comprehensive evaluation using membership function analysis was performed to assess seedling recovery under different treatment conditions.

The results showed that, compared with the control group, the combined treatments significantly improved the survival rates of both ZN and ZJN seedlings. ZN exhibited a significant increase in new leaf production, whereas ZJN showed no such increase. Chlorophyll and carotenoid contents increased significantly across treatments, with more pronounced effects observed in the ZN1 and ZJN1 groups. The ratios of (GA3+ZR+IAA)/ABA and IAA/ABA were also significantly elevated. While no significant differences were detected among different concentration treatments in ZN, ZJN1 showed significantly higher values than ZJN2. Soluble protein and soluble sugar contents decreased in treated seedlings. Membership function comprehensive evaluation revealed the following rankings for transplant survival performance: ZN1 > ZN2 > CK for *P. zhennan* and ZJN1 > ZJN2 > CK for *P. chekiangensis*. In conclusion, the combined super-absorbent polymer and ABT treatment promoted the survival of bare-root ZN and ZJN seedlings, with the ZN1 and ZJN1 concentrations demonstrating optimal efficacy.

Keywords: *Phoebe zhennan*, *Phoebe chekiangensis*, super-absorbent polymer, ABT, bare-root transplanting, membership function analysis

Introduction

Phoebe zhennan and *P. chekiangensis* are species in the Lauraceae family, genus *Phoebe*, with limited natural distribution in Guizhou, Sichuan, Chongqing, Hubei, and other regions of China. Classified as endangered species, these trees

feature straight trunks, elegant forms, dense foliage, and evergreen leaves, offering high ornamental value and significant potential for landscape applications [?]. Their high-quality timber is resistant to decay and insect damage, and emits a subtle fragrance, making it highly sought after in commercial markets for premium wood products. Although recent progress has been made in improving transplant survival of landscape tree species—for instance, systematic reviews have summarized key factors and strategies [?], and Huang et al. (2013) explored the effects of microbial inoculants on *Euscaphis konishii* seedlings—research on enhancing bare-root transplant survival of *P. zhennan* and *P. chekiangensis* remains limited. Studies on shortening their post-transplant recovery period are particularly scarce [?], with these technical deficiencies severely restricting the development, promotion, and application of these valuable species. Consequently, innovative methods to improve transplant survival are urgently needed to meet market demands.

During bare-root transplanting and recovery, plants face multiple stressors including root damage, water loss, mechanical injury, and sudden changes in rhizosphere conditions, collectively termed “transplanting stress” [?]. These stressors disrupt plant homeostasis, leading to decreased chlorophyll content and accumulation of soluble sugars and proteins [?], ultimately causing plant death, growth arrest, or abnormal development that severely limits post-transplant survival and recovery [?]. Monitoring physiological and biochemical indicators during bare-root transplanting can therefore provide valuable insights into plant health status. Previous research has employed this approach extensively: studies on two-year-old sweet cherry bare-root seedlings treated with different growth regulators examined survival rates, growth metrics, chlorophyll content, and leaf water content to identify optimal treatments [?], while field experiments assessed the effects of seedling strengtheners on rapeseed transplant survival by measuring soluble sugar and protein contents [?]. Similarly, evaluating chlorophyll content, soluble sugars, soluble proteins, and endogenous hormone levels can provide a comprehensive assessment of recovery in transplanted *P. zhennan* and *P. chekiangensis* seedlings.

Recent studies have demonstrated that super-absorbent polymers and ABT can improve transplant survival. For example, super-absorbent polymer treatment enhanced stolon germination rate, germination potential, germination index, and root growth in bermudagrass seedlings, significantly promoting transplant survival [?]. Cui et al. (2011) found that super-absorbent polymer treatment not only improved rooting, seedling growth, and quality in fruit trees like apple but also reduced production costs. Likewise, ABT alone can promote survival of landscape tree seedlings: Zhu et al. (2017) observed that ABT No.1 promoted rooting in ginkgo seedlings, though rooting rate initially increased then decreased with rising concentrations. Moreover, combined super-absorbent polymer and ABT treatments have shown superior effects. Liu et al. (2018) reported that this combination significantly increased transplant survival of *Phoebe bournei* seedlings, while Sun et al. (2012) demonstrated significantly improved survival of spring and autumn transplants of *Ceratoides arborescens*

in arid regions. These findings collectively indicate protective effects of combined super-absorbent polymer and ABT treatments on plant survival after transplanting.

However, the specific effects of combined super-absorbent polymer and ABT treatments on bare-root seedlings of *P. zhennan* and *P. chekiangensis* remain unclear. This study therefore investigates the impacts of different concentrations of combined super-absorbent polymer and ABT treatments on the survival and recovery of bare-root *P. zhennan* and *P. chekiangensis* seedlings. By measuring survival rates, new leaf numbers, ten physiological indicators, and conducting membership function analysis, we quantitatively evaluate treatment effects to provide theoretical support and practical guidance for post-transplant recovery and promote wider application of these species.

1.1 Experimental Materials

All experimental plant materials were obtained from the Taihu Science and Technology Park of Yangtze University in Jingzhou, Hubei Province (111°15' - 114°05' E, 29°26' - 31°37' N). The study utilized four-year-old *P. zhennan* and *P. chekiangensis* seedlings with uniform growth vigor (average height: 72.3 ± 0.3 cm; average ground diameter: 3.85 ± 0.24 cm). The region features a north subtropical monsoon humid climate with an average annual temperature of 15.9-16.6 °C, a frost-free period of 242-263 days, annual sunshine duration of 1,800-2,000 hours, and annual precipitation of 1,100-1,300 mm. The favorable site conditions are well-suited for *Phoebe* species growth.

On March 17, 2017, 300 seedlings each of *P. zhennan* (ZN) and *P. chekiangensis* (ZJN) were collected. Prior to transplanting, all seedlings were processed by removing damaged and lower leaves (remaining leaves were halved) and eliminating broken or diseased roots. Both species were randomly divided into three groups of 100 seedlings each, with specific treatments detailed in Table 1. A blank treatment served as the control (CK).

Table 1 Experimental groups and treatments

Treatment Group	Super-absorbent polymer ($\text{g} \cdot \text{L}^{-1}$)	Adhesion agent ($\text{g} \cdot \text{L}^{-1}$)	Muddy water (v:v)
ZN1, ZJN1			
ZN2, ZJN2			
ZNCK, ZJNCK			

Seedlings in each group were treated by immersing their roots in the prepared

mud reagent for 30 minutes. After immersion, roots were wrapped in plastic film, and seedling stems were coated with muddy water to maintain moisture before transport to the nursery for transplanting. Stem coating with corresponding muddy water was repeated weekly until September 2017, when the local dry season ended. Seedlings received thorough watering immediately after transplanting, followed by uniform daily irrigation without additional management. Final sampling was conducted on November 5, 2018. Additionally, 100 seedlings each of ZN and ZJN were grown in the same nursery without any treatment, designated as ZN and ZJN groups. Transplanted seedlings were established in the *Phoebe* germplasm resource base at Yangtze University's Agricultural Industry Science and Technology Park, a location with similar environmental conditions to the material source site, thereby minimizing environmental variation.

During sampling, healthy, disease-free current-year leaves were collected from each group, rapidly frozen in ice boxes, and transferred to an ultra-low temperature freezer at -80°C for long-term storage until analysis. All measurements were completed within one week of sampling.

1.3.1 Survival Rate and New Leaf Count of Transplanted Seedlings

Survival numbers and new leaf production were determined through direct observation and counting for each ZN and ZJN group.

1.3.2 Physiological Index Detection in Transplanted Seedlings

Chlorophyll and carotenoid (CAR) contents were determined using spectrophotometry [?], with calculations following the methods of Zhang and Jiang (2014). Soluble sugar and soluble protein contents were measured using the anthrone colorimetric method and Coomassie brilliant blue G-250 staining, respectively [?]. Endogenous hormone contents were analyzed using high-performance liquid chromatography at the Horticulture and Landscape Architecture College of Yangtze University, with parameters set according to the method described by Wang et al. (2013) [?].

1.4 Data Statistics and Processing

All data were analyzed and plotted using Origin 2019. Statistical comparisons were performed using Duncan's multiple range test, with $P < 0.05$ considered statistically significant. Experimental data are expressed as mean \pm standard error. To minimize errors from individual plant variation and human factors and improve data reliability, this study introduced the difference coefficient (R) and membership function (U) to comprehensively evaluate leaf physiological indices of *P. zhennan* and *P. chekiangensis* under combined super-absorbent polymer and ABT treatments.

The difference coefficient was calculated as:

$$R_{ij} = \left| \frac{X_{ij} - N_j}{N_j} \right|$$

where R is the difference coefficient for indicator j in group i , X is the measured value, and N is the value for normal plants.

The membership function was used to comprehensively evaluate physiological indicators and rank the difference coefficients relative to normal plant status:

$$U_{ij} = \frac{X_{ij} - X_{i \min}}{X_{i \max} - X_{i \min}}$$

where U is the membership function value, X is the measured value, and X_{\min} and X_{\max} are the minimum and maximum values for each indicator, respectively. The average membership function value for each species was calculated for comparative analysis.

2.1 Combined Super-Absorbent Polymer and ABT Treatment Enhanced Survival Rate and New Leaf Production

As shown in Figure 1 [Figure 1: see original paper], the survival rate and new leaf count of *P. zhennan* seedlings in the ZN1 treatment group exceeded those in the ZN2 group and were significantly higher than the CK group, indicating that combined super-absorbent polymer and ABT treatment at both concentrations promoted post-transplant survival and growth, with more pronounced effects at the ZN1 level. For *P. chekiangensis*, the ZJN1 treatment group exhibited significantly higher survival rates compared with ZJN2 and CK groups, though no significant differences in new leaf numbers were observed among the three treatments, suggesting that the combined treatment primarily influenced survival rather than leaf production (Figure 1). Overall, *P. zhennan* seedlings demonstrated higher survival rates and new leaf numbers than *P. chekiangensis* under identical treatments.

Note: Different lowercase letters indicate significant differences among treatments ($P < 0.05$). The same notation applies below.

Figure 1 Survival rate (A) and average new leaves (B) of bare-root transplanted *Phoebe zhennan* (ZN) and *P. chekiangensis* (ZJN) under combined super-absorbent polymer and ABT treatment.

2.2 Combined Treatment Enhanced Photosynthetic Pigment Content

As illustrated in Figure 2 [Figure 2: see original paper], both *P. zhennan* and *P. chekiangensis* seedlings in the ZN1 and ZJN1 treatment groups showed significantly higher chlorophyll and carotenoid contents compared with ZN2 and ZJN2 groups, respectively, and all treatment groups exceeded the CK group. These results indicate that combined super-absorbent polymer and ABT treatment enhanced photosynthesis in transplanted seedlings at both concentrations, with more significant effects observed at the ZN1 and ZJN1 levels. Additionally, *P. zhennan* seedlings consistently exhibited higher photosynthetic pigment contents than *P. chekiangensis* under equivalent treatments.

Figure 2 Chlorophyll (A) and carotenoid (B) contents in bare-root transplanted *Phoebe zhennan* (ZN) and *P. chekiangensis* (ZJN) seedlings under combined super-absorbent polymer and ABT treatment.

2.3 Combined Treatment Influenced Endogenous Hormone Levels

This study measured four major plant hormones in treated *P. zhennan* and *P. chekiangensis* seedlings. Considering hormonal synergism, the ratios of combined GA3, IAA, and ZR levels to ABA, as well as the IAA/ABA ratio, were used as primary reference indicators. As shown in Figure 3 [Figure 3: see original paper], *P. zhennan* exhibited no significant differences in (GA3+ZR+IAA)/ABA and IAA/ABA ratios between ZN1 and ZN2 treatments, though both were significantly higher than the CK group. In contrast, *P. chekiangensis* seedlings in the ZJN1 treatment group displayed significantly higher hormone ratios compared with the ZJN2 group. These findings indicate that both ZN1 and ZN2 treatments effectively enhanced growth-promoting endogenous hormone secretion in *P. zhennan*, whereas only the ZJN1 concentration promoted hormonal balance in *P. chekiangensis*. No differences in endogenous hormone ratios were observed between species within the same treatment groups.

Figure 3 Ratios of endogenous hormones (GA3+ZR+IAA)/ABA (A) and IAA/ABA (B) in bare-root transplanted *Phoebe zhennan* (ZN) and *P. chekiangensis* (ZJN) under combined super-absorbent polymer and ABT treatment.

2.4 Combined Treatment Reduced Osmotic Substance Content

As depicted in Figure 4 [Figure 4: see original paper], ZN1 and ZJN1 treatments significantly reduced the contents of both osmotic substances compared with the CK group, while ZN2 and ZJN2 treatments also decreased soluble sugar and soluble protein levels, albeit non-significantly. These results demonstrate that ZN1 and ZJN1 concentrations effectively mitigated transplanting stress in both

P. zhennan and *P. chekiangensis*. However, no significant differences in osmotic substance contents were observed between the two species across all treatments, indicating similar stress responses to bare-root transplanting.

Figure 4 Effects of combined super-absorbent polymer and ABT treatment on soluble sugar (A) and soluble protein (B) contents in bare-root transplanted *Phoebe zhennan* (ZN) and *P. chekiangensis* (ZJN) seedlings.

2.5 Difference Coefficients of Physiological Indices After Combined Treatment

To better assess recovery of *P. zhennan* and *P. chekiangensis* seedlings after combined treatment, difference coefficients (R) were calculated to evaluate changes in physiological and endogenous hormone indicators. As shown in Table 2, for *P. zhennan*, all indices except ZR and IAA exhibited R values following the trend ZN1 < ZN2 < ZNCK, indicating that ZN1-treated seedlings displayed physiological status closest to normal plants.

Similar patterns were observed in *P. chekiangensis*, where all indices except ZR showed R values following the order ZJN1 < ZJN2 < ZJNCK (Table 2). These results demonstrate that the ZJN1 concentration of combined super-absorbent polymer and ABT treatment was most beneficial for physiological recovery of *P. chekiangensis* bare-root transplants.

Table 2 Restitution coefficient (R) of physiological indices in leaves of *Phoebe zhennan* and *P. chekiangensis* under combined super-absorbent polymer and ABT treatment

Index	Difference coefficient (R)		
	<i>P. zhennan</i> ZNCK	<i>P. chekiangensis</i> ZN2	ZN1
IAA/ABA			
Hor/ABA			
...			

Note: SS = soluble sugar; SP = soluble protein; Hor = (GA3+ZR+IAA)/ABA; CHL = chlorophyll content; CAR = carotenoid content; GA3 = gibberellic acid; ABA = abscisic acid; ZR = zeatin; IAA = auxin; ZN = *Phoebe zhennan*; ZJN = *P. chekiangensis**

2.6 Comprehensive Evaluation of Physiological Indices After Combined Treatment

Based on the difference coefficient R values, fuzzy mathematics membership function analysis was applied to comprehensively evaluate various indicators and further explore the effects of combined treatment. As shown in Table 3, *P. zhennan* seedlings in the ZN1 treatment exhibited the lowest membership function U values, followed by ZN2, with the CK group showing the highest U values. This indicates that ZN1 treatment produced the best recovery in transplanted *P. zhennan* seedlings, with ZN2 treatment ranking second.

For *P. chekiangensis*, ZJN2 treatment showed the lowest U values, followed by ZJN1, while the CK group displayed the highest values (Table 4). These results suggest that ZJN2 concentration was most favorable for post-transplant recovery of *P. chekiangensis*, with ZJN1 providing partial recovery and CK showing the weakest restoration.

Furthermore, membership function values for *P. zhennan* were substantially lower than those for *P. chekiangensis* at both treatment levels ($ZN1 < ZJN1$, $ZN2 < ZJN2$), indicating that *P. zhennan* exhibited stronger recovery capacity under combined super-absorbent polymer and ABT treatment.

Table 3 Comprehensive indicator value of subordination function U of combined treatment in *Phoebe zhennan*

<i>P. zhennan</i>	Membership function value U			
	U(1)	U(2)	U(3)	Average
ZNCK				
ZN2				
ZN1				

Table 4 Comprehensive indicator value of subordination function U of combined treatment in *Phoebe chekiangensis*

<i>P. chekiangensis</i>	Membership function value U			
	U(1)	U(2)	U(3)	Average
ZJNCK				
ZJN2				
ZJN1				

Discussion

Phoebe zhennan and *P. chekiangensis* are nationally protected Grade II plants and endangered species in China. Previous studies have demonstrated consis-

tent protective effects of super-absorbent polymers and ABT on transplant survival. Li (2013) confirmed that combined treatment promoted post-transplant growth and cold resistance in magnolia, willow, and juniper. Additionally, Liu et al. (2018) reported that combined ABT and super-absorbent polymer treatment significantly improved transplant survival of *Phoebe bournei*, a congeneric species. Consistent with these findings, our study demonstrates that combined treatment enhanced survival rates of bare-root *P. zhennan* and *P. chekiangensis* seedlings, promoted post-transplant growth and recovery, and showed concentration-dependent effects. These results collectively indicate that combined super-absorbent polymer and ABT treatment provides protective effects for bare-root seedlings of these valuable species, facilitating post-transplant recovery and growth.

During transplanting, inevitable root damage triggers changes in various physiological indicators, making their monitoring essential for assessing plant status. Previous research has shown that transplant stress reduces leaf chlorophyll and carotenoid contents during early stages [?], reflecting adverse stress impacts. Effective protective agents should therefore enhance photosynthetic pigment levels. Studies have demonstrated that super-absorbent polymer treatment increased chlorophyll content in transplanted gerbera in a concentration-dependent manner, with ABT also promoting chlorophyll production [?]. Our findings align with these results, showing significant post-transplant declines in chlorophyll and carotenoid contents in both species, indicating stress-induced damage, while combined treatment restored photosynthetic pigment levels and partially recovered photosynthetic capacity.

Endogenous hormones play indispensable roles throughout plant development, maintaining dynamic balance and synergistic interactions. These hormones are sensitive to environmental changes, with stress conditions typically increasing growth-inhibiting ABA while decreasing growth-promoting IAA and ZR, consequently reducing (GA3+ZR+IAA)/ABA and IAA/ABA ratios [?]. Chen et al. (2013) observed elevated ABA/(GA3+ZR+IAA) ratios and decreased IAA/ABA ratios in transplanted *Camellia oleifera* seedlings under drought stress, indicating growth limitation. Similarly, drought stress substantially increased ABA content in apple roots [?]. Unfortunately, research on endangered *Phoebe* species remains limited, particularly regarding post-transplant endogenous hormone dynamics. Our study reveals significant declines in both hormone ratios after bare-root transplanting, with combined treatment restoring these ratios, especially under ZN1 and ZJN1 concentrations. These findings demonstrate that super-absorbent polymer and ABT treatment effectively restores growth in transplanted *P. zhennan* and *P. chekiangensis*, with ZN1 and ZJN1 concentrations providing superior protection.

Importantly, plants represent dynamic systems where single factors cannot accurately reflect physiological status. Comprehensive evaluation using difference coefficients and membership functions enables robust assessment of multi-indicator effects on plant characteristics. For example, Fu et al. (2006) used membership

functions to evaluate light adaptation in transplanted magnolia, while Wang et al. (2013) assessed drought resistance in different *Lespedeza* varieties. These studies demonstrate the persuasive power of membership function analysis for evaluating comprehensive indicator effects. Our membership function analysis confirms that combined treatment benefited recovery of both *P. zhennan* and *P. chekiangensis* bare-root transplants, supporting previous conclusions. Notably, *P. zhennan* exhibited lower membership function values than *P. chekiangensis*, indicating stronger adaptability to transplant stress under identical conditions. Based on these results, we recommend prioritizing *P. zhennan* for landscape applications to improve transplant survival and reduce landscaping costs.

In conclusion, combined super-absorbent polymer and ABT treatment significantly enhances survival and recovery of *P. zhennan* and *P. chekiangensis* bare-root seedlings. While this study provides preliminary insights into the survival-promoting and recovery-enhancing mechanisms of combined treatment, the specific modes of action warrant further investigation.

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